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# SOIL SCIENCE

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SOIL · CHEMISTRY · AND · SOIL · BIOLOGY

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in nature and was originally believed to be hydrolyzed microbiologically only by the bacteria. A number of later workers (46, 48, 50, 69, 75, 91, 103, 118, 120) have shown, however, that cellulose, either in the pure form or in the natural or crude state found in plant residues such as straw, roots, or bagasse, is hydrolyzed by filamentous fungi and used by them as a source of energy. It has also been shown (118) that nitrogen is necessary in this process for the metabolism of the organisms. Lignin is probably one of the most resistant substances found in organic residues. Waksman and Tenney (119) found the lignin unattacked by most soil microorganisms and were able to recover it almost quantitatively at the end of a 35-day incubation period. On the other hand, von Schrenk (94) has shown that even lignin is hydrolyzed and destroyed by the white rot fungus *Polyporus juniperinus*. Other substances such as  $\beta$ -methylglucoside (36) and higher alcohols (76) have been shown to be used as sources of energy by fungi.

Natural organic materials in the form of plant residues probably contain many if not all of these carbonaceous substances which are used as energy for the growth of the fungi. Wheat straw, for example, according to Waksman (113), contains 21.67 per cent pentosans, 34.27 per cent cellulose, and 21.21 per cent lignin. It also contains water-soluble carbonaceous materials, and so furnishes an excellent source of energy for the fungi.

#### *Relation of fungi to nitrogen*

Soil microorganisms are concerned with nitrogen in three ways: first, the assimilation of free nitrogen; second, the transformation of organic to inorganic forms; third, the use of combined nitrogen in the production of cellular or mycelial substances.

*Fixation of free nitrogen.* Among the early workers on the fixation of free nitrogen by soil fungi was Lipman (66) who reported a weak fixing power for species of *Aspergillus* and *Penicillium*. Later workers (17, 42, 63), however, seem to be of the opinion that with the possible exception of *mycorrhiza*, there is no fixation of atmospheric nitrogen by soil fungi. Although Duggar and Davis (37) concurred in the general opinion of the latter workers their work seems to indicate that *Phoma betae* has a weak nitrogen fixing power.

*Ammonification.* The power of soil fungi to hydrolyze nitrogenous organic materials, especially the proteins and amino compounds found in the organic matter, has been investigated by a number of workers (1, 62, 70, 108, 110, 116) who have shown this property to be very general among the fungi. A great many of the fungi have the power to break down the proteins and to liberate amino acids, or ammonia, or both. Thus the soil fungi play a very important rôle in this stage of the mineralization process.

*Use of nitrogen.* It is generally conceded by most investigators (9, 12, 19, 60, 82, 118) that nitrogen is not only used by the fungi in their growth but is also a necessary element in the formation of their substance. This nitrogen they may use in the inorganic form as nitrate, nitrite, or ammonia, or in the

various amino forms. Reduction of nitrogen has been shown, but there is very little evidence of denitrification or the loss of combined nitrogen from fungous activities. The use of nitrogen by fungi is always associated with the use of energy material and it has been pointed out (8, 16, 19, 48, 87, 98, 120) that this nitrogen, if not an absolute necessity in the decomposition of the cellulosic energy material, is an aid in this process and hastens the utilization of the cellulose by the fungi. The amount of nitrogen necessary in this decomposition process has been estimated by Waksman and Heukelekian (47, 117, 118) to be 1 part of nitrogen for every 30 to 50 parts of energy material in the form of cellulose used by the fungi. This nitrogen is used in the building of mycelial tissue, and the amount consumed will vary somewhat with the organisms and its environmental conditions.

The result of this use of nitrogen in relation to the utilization of energy material by microorganisms is seen under field conditions in the depression of nitrate nitrogen when the energy material of the soil is increased. A number of workers (5, 68, 74, 85, 95, 97, 99) have reported that when energy material in the form of plant residue, either tops or roots, is incorporated into the soil there is an immediate depression in the nitrate content. At first toxins were thought to be the cause of this depression but later investigations (13, 20, 67, 106) seem to indicate that it is only a disturbance of the energy-nitrogen balance and that the nitrogen is not lost, but is used by the soil microorganisms.

As a brief summary it may be stated that the soil fungi use the carbonaceous materials of the soil as energy for their growth and the soil nitrogen in building their mycelial tissue. If the ratio of energy material to nitrogen is decreased, soluble nitrogen is liberated, but if this ratio is increased, these organisms use up available nitrogen. It is then easy to believe that soil fungi are largely responsible for the rapid depression of mineral nitrogen when the energy supply of the soil is increased.

#### COMPOSITION OF FUNGOUS MYCELIUM<sup>2</sup>

The composition of fungus tissue has been the subject of investigation for over a century. Perhaps the outstanding single contribution is the monograph by Zellner (136) in which he has compiled much of this material, especially from the earlier works. The greater part of this information, however, is of a general qualitative nature and only a limited amount of quantitative data is given. The reason for this may be seen in the fact that not only the quantitative but even the qualitative composition of fungous tissue depends much upon the environmental conditions of the organism during its period of growth. Zellner has shown that as a group the fungi contain about the

<sup>2</sup> While this article was in press, a paper appeared by R. C. Thomas [Composition of fungus hyphae I. The *Fusaria*. *Amer. Jour. Bot.* 15: 537-547. (1928)] dealing with the composition of the hyphae of species of *Fusaria*. This author thinks that the outer covering of the hyphae is made up of a protein-pectic compound and a cellulose-fatty-acid complex with a basic skeleton of chitin.

same groups of compounds, with the possible exception of cellulose and lignin, as the higher plants. In the nitrogenous group they contain proteins of various kinds, amino acids, amines, basic nitrogenous and purine substances, urea, and lecithin, as well as certain toxic substances of an alkaloid nature. In the carbonaceous group may be mentioned glucose, trehalose, glycogen, pentosans, mycodextran, paraisodextran, inulin, viscosin, chitin, organic acids, fats, higher alcohols, and a cellulosic carbonaceous material of unknown composition.

### *Nitrogenous portion*

Because of variability in culture media, methods of analysis and species employed, the analytical data available for fungous tissue are more or less unsatisfactory from a quantitative standpoint. The carbon content of fungous tissue is rather constant but its content of nitrogen is quite variable. Analyses reported by Sieber (96), Mazé (73), Peterson, Fred, and Schmidt (80), Heukelekian and Waksman (48), and the writer are given in table 1.

These data indicate that the ratio of carbon to nitrogen of the mycelium, although rather stable and usually falling between 7 and 10 to 1, depends somewhat on the carbon-nitrogen ratio of the medium upon which the mycelium is grown and may be greater than 20 to 1 where this ratio in the medium is very high. Waksman and Heukelekian (118) reported 45 per cent of carbon in the dry mycelium and a nitrogen content of from 4 to 8 per cent depending on the nitrogen content of the medium. From the results given, it appears that the nitrogen content of the mycelium decreases with a decrease in the nitrogen supply until it reaches about 2 per cent, after which a further decrease in the supply of nitrogen results in a decreased production of mycelium. The results of other workers are in accord with the data already given.

The nature of the nitrogenous substances in fungous tissue is not definitely known. The following estimates made by Winterstein and Reuter (132) on the tissue of *Boletus edulis* give some idea as to the kinds and amounts of the various substances:

	<i>per cent</i>
Moisture .....	10
Ether extract .....	4
(Fat 3.25 per cent, coleslerin 0.5 per cent and lecithin)	
Alcohol extract.....	12
(Trehalose 3 per cent, sugar, lecithin, trimethyl-histidine, adenine, guanine, hypoxanthine, choline, alanine, leucine, purine bodies, bases, etc. 9 per cent)	
Water extract .....	28
(Glycogen 5 per cent, trehalose, purine bodies, bases, amino acids, ash, etc., 23 per cent)	
Residue.....	46
(Protein 30 per cent, amorphous carbohydrates [paraisodextran] 10 per cent, and chitin 6 per cent)	

In *Agaricus campestris*, Winterstein and associates (133) estimated that 51.9 per cent of the total nitrogen is protein nitrogen, 7.8 per cent basic nitro-

Relation of carbon and nitrogen in fungous media

INVESTIGATOR	ORGANISM	CULTURE AGE days	MEDIA			MYCELIUM		
			Energy material	Nitrogen material	C/N ratio	Carbon per cent	Nitrogen per cent	C/N ratio
Sieber (1881)	<i>Penicillium</i> sp. and <i>Aspergillus glaucus</i>	75	Sugar	Gelatin	4.75	45.95	5.32	8.64
	<i>Penicillium</i> sp. and <i>Aspergillus glaucus</i>	75	Sugar	NH <sub>4</sub> Cl	9.60	46.03	5.34	8.62
Mazé (1902)	<i>Eurotopsis gayana</i>	5	Sucrose	....	21.05	51.67	4.48	11.30
	<i>Eurotopsis gayana</i>	9	Alcohol	....	9.01	50.45	5.55	9.10
Peterson, Fred and Schmidt (1922)	<i>Eurotopsis gayana</i>	8	Glycerol	....	19.55	48.89	4.67	10.47
	<i>Eurotopsis gayana</i>	8	Lactic acid	....	20.00	51.51	4.73	10.89
	<i>Aspergillus niger</i>	7	Xylose	NH <sub>4</sub> NO <sub>3</sub>	22.86	44.5	4.5	9.90
	<i>Aspergillus niger</i>	9	Xylose	NH <sub>4</sub> NO <sub>3</sub>	22.86	46.9	4.6	10.20
	<i>Aspergillus niger</i>	14	Xylose	NH <sub>4</sub> NO <sub>3</sub>	22.86	46.0	4.2	10.95
	<i>Aspergillus niger</i>	28	Xylose	NH <sub>4</sub> NO <sub>3</sub>	22.86	45.4	4.2	10.81
	<i>Aspergillus</i> sp.	14	Xylose	NH <sub>4</sub> NO <sub>3</sub>	22.86	47.3	4.7	10.07
	<i>Penicillium glaucum</i>	9	Xylose	NH <sub>4</sub> NO <sub>3</sub>	22.86	50.7	5.7	8.90
	<i>Penicillium glaucum</i>	14	Xylose	NH <sub>4</sub> NO <sub>3</sub>	22.86	46.3	5.0	9.26
	<i>Penicillium glaucum</i>	29	Xylose	NH <sub>4</sub> NO <sub>3</sub>	22.86	49.7	5.9	8.42
This work (1928)	<i>Aspergillus oryzae</i>	14	Sucrose	NH <sub>4</sub> NO <sub>3</sub>	6.00	40.48	6.42	6.30
	<i>Aspergillus oryzae</i>	14	Sucrose	NH <sub>4</sub> NO <sub>3</sub>	30.00	41.10	3.83	10.70
	<i>Aspergillus oryzae</i>	14	Sucrose	NH <sub>4</sub> NO <sub>3</sub>	150.00	39.55	1.89	20.90
						mgm.	mgm.	
	<i>Trichoderma</i> sp. in solution	17	Cellulose	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	10.95	54.90	12.3	4.46
	<i>Trichoderma</i> sp. in solution	24	Cellulose	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	10.95	78.5	18.2	4.31
	<i>Trichoderma</i> sp. in solution	31	Cellulose	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	10.95	138.2	22.2	6.22
	<i>Trichoderma</i> sp. in solution	38	Cellulose	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	10.95	138.3	23.7	5.83
Heukelekian and Waksman (1925)	<i>Trichoderma</i> sp. in sand	7	Cellulose	NH <sub>4</sub> NO <sub>3</sub>	11.40	128.9	18.9	6.80
	<i>Trichoderma</i> sp. in sand	14	Cellulose	NH <sub>4</sub> NO <sub>3</sub>	11.40	283.3	27.4	10.30

## STATEMENT

OF THE OWNERSHIP, MANAGEMENT, CIRCULATION, ETC., REQUIRED BY THE ACT OF  
CONGRESS OF AUGUST 24, 1912,

of Soil Science, published monthly at New Brunswick, N. J., for April 1, 1916.

*State of New Jersey,*

*County of Middlesex,*

Before me, a Notary Public in and for the State and county aforesaid, personally appeared Jacob G Lipman, who, having been duly sworn according to law, deposes and says that he is the publisher of SOIL SCIENCE and that the following is, to the best of his knowledge and belief, a true statement of the ownership, management, etc., of the aforesaid publication for the date shown in the above caption, required by the Act of August 24, 1912, embodied in section 443, Postal Laws and Regulations, to wit:

1 That the names and addresses of the publisher, editor, managing editor, and business managers are:

Publisher, Jacob G Lipman, New Brunswick, N J

Editor, Jacob G Lipman, New Brunswick, N J

Managing Editor, Carl R. Woodward, New Brunswick, N. J.

Business Manager, Carl R. Woodward, New Brunswick, N. J.

2. That the owners are:

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(Signature) JACOB G. LIPMAN.

Sworn to and subscribed before me this 12th day of June, 1916.

IRVING E. QUACKENBOSS,

Notary Public, Middlesex County, New Jersey.





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## ERRATA

- Page 17, line 8 from bottom, "nittrate" should read "nitrate."
- Page 48, 3rd page following, legend opposite Plate II, last line, "as" should read "at."
- Page 94, reference (10), title of article should read "Untersuchungen über das Verhalten des Ammoniakstickstoff in gekalkten und ungekalkten Boden."
- Pages 104, 108, 109-128, "Czapeck" and "Czapeck's" should read "Czapek" and "Czapek's," respectively.
- Page 110, line 17 from bottom, "violaceus" should read "*violaceus-ruber*."
- Page 125, line 11, omit "33."
- Page 129, line 21, "*albotratus*" should read "*alboatrus*."
- Page 131, Table V, "violaceons-ruber" should read "*violaceus-ruber*," and "violaceons-Caeseri" should read "*violaceus-Caeseri*."
- Page 134, 3rd page following, legend opposite Plate II, "*Act. albotratus*" should read "*Act. alboatrus*."
- Page 151, reference (9) should read "Koch, G. P. 1915. Activity of soil protozoa. *In Jour. Agr. Research*, v. 5, no. 11, p. 477-488."
- Page 180, line 14, "*bacillus fluorescens liquifi*" should read "*Bacillus fluorescens liquefaciens*."
- Page 182, line 14, "1 per cent" should read "0.03645 per cent."
- Page 188, line 4, "*bacillus fluorescens liqui*" should read "*Bacillus fluorescens liquefaciens*."
- Page 232, line 1, "Table XXXXIV" should read "Table XXXIV."
- Page 274, reference (7), "Lyon, T. A." should read "Lyon, T. L.," and references (11) and (12), "Russell, E. H." should read "Russell, E. J."
- Page 325, Table VII, "January 17—February 20" should read "January 17—January 20." Also, insert "November 24—January 7" under line reading "Group B.—Formation of Nitrates."
- Page 386, legend of figure 2, "or" should read "of."
- Page 392, Table VI, heading of 1st column under "Dried Blood Series" should read "Incr. 1 c.c. over 0.2 c.c.", of 2nd column, "Incr. 5 c.c. over 1.0 c.c.", and heading of 1st column under "Cottonseed Meal Series" should read "Incr. 1 c.c. over 0.2 c.c."
- Pages 381-403, "The Inoculation and Incubation of Soil Fungi," by N. Kopeloff. Throughout this article, "*Zygorrhyncus*" should read "*Zygorhynchus*" (preferable form).

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Dedicated to the Memory  
of  
Eugene Waldemar Hilgard, Ph.D., LL.D.  
Zweibrücken, Bavaria, January 5, 1833  
Berkeley, California, January 8, 1916

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EUGENE WOLDEMAR HILGARD, Ph.D., LL.D., Late Director Emeritus of the Agricultural Experiment Station of California, and Professor Emeritus of Agriculture of the University of California.

## EUGENE WOLDEMAR HILGARD

Zweibrücken, Bavaria, Jan. 5, 1833—Berkeley, California, Jan. 8, 1916

Having been granted the vision and the strength to achieve, Doctor Hilgard labored humbly and gratefully to the very end, in his chosen field of science. Born at Zweibrücken, Bavaria, in 1833, he studied at Zürich, Freiberg and Heidelberg and as a young man came to the United States for a long term of faithful and distinguished service. In 1855 he was appointed chemist of the Smithsonian Institute, but resigned in the following year to accept service with the State of Mississippi. He remained there until 1873 in the capacity successively, of assistant state geologist, state geologist and professor of chemistry. He then accepted an appointment as professor of geology and natural history at the University of Michigan. Ill health compelled him to resign in 1875 and to seek a milder climate which he found in California. There he studied, taught; and inspired many men for more than forty years. In his capacity as professor of agriculture and director of the agricultural experiment station he helped to build up the agriculture of a great commonwealth; but even more than that, he made important contributions to soil physics and soil chemistry and aided thus in the building of the foundations of the science of soils.

Not possessed of a rugged physique he was nevertheless a tireless worker and always eager to help. His responsibilities at the university did not deter him from important service elsewhere. As special agent of the Tenth Census, as one of the officials of the Northern Transcontinental Survey, and as chairman of the Committee on Agriculture in Arid Regions he rendered service of national or international scope. In recognition of this service and of his numerous contributions to agricultural geology, plant physiology, soil physics and soil chemistry, he was awarded the Liebig Medal by the Munich Academy in 1894; a diploma and gold medal at the Paris Exposition in 1900; was elected honorary member of several scientific societies, and was given the honorary degree of LL.D. by the Universities of Mississippi, Michigan and Columbia. But while the world of science knew of his work and honored him for it, it was the peculiar privilege of his associates to find inspiration in his kind and helpful nature, in his catholic sympathies, in his clear vision and in his unflinching devotion to truth.



# SOIL SCIENCE

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## INTRODUCTORY

Specialization must follow expansion in every field of knowledge. The growing mass of facts, observations and deductions must be classified, divided and subdivided. They must be made ready for the far sweep and insight of genius, ready for those broad generalizations that will fit the scattered and apparently unrelated parts into a symmetrical design. Without this preliminary gathering and grouping of facts, a task often tedious, at times seemingly unprofitable, the progress of science would be halted and the understanding of the great laws of nature would be dimmed.

Specialized technical publications are the necessary outcome of specialization in research. When investigation is carried on in a field of science but meagerly explored, the findings may be recorded in journals not specifically devoted to those problems. But as the data accumulate and the number of workers in a given field is increased the need becomes more urgent for a specific medium. That this need is now being felt in the field of soil research is attested to both by the large number of projects under investigation and the many specialists in the domains of soil physics, soil chemistry and soil biology. Scores of technical papers on the various phases of soil fertility appear in the course of each year as station research bulletins or in publications like the *Journal of Industrial and Engineering Chemistry*, the *Journal of the American Society of Agronomy*, the *Journal of Agricultural Research*, the *Botanical Gazette*, *Science* and a number of others. Many American contributions to soil science appear also in European journals, among them *Centralblatt für Bakteriologie und Parasitenkunde*, *Zweite Abteilung*, *Internationale Mitteilungen für Bodenkunde* and the *Journal of Agricultural Science*. It is evident, therefore, that under existing conditions the soil investigator is put to much inconvenience in keeping before him all of the more important papers in soil research. Moreover, he finds it increasingly difficult to secure the prompt publication of his own papers in journals whose contributions cover a wide range of scientific activity. Not infrequently six

months or more must elapse between the writing of a paper and of its appearance in print.

In planning for the publication of *SOIL SCIENCE* the Editor was guided by the wish to facilitate the bringing to light the results of soil research. He felt encouraged to believe that the new journal would help to conserve the time and the energies of his fellow students of soils; that it would provide for a more direct contact among men interested in the same problems; and that it would lead to a broader outlook on the entire field of soil fertility. There need be no fear that *SOIL SCIENCE* will in any way impair the value or usefulness of other technical journals. From year to year there is a greater number research papers made available for publication in all the fields of science. As time goes on the older journals find it expedient to draw the lines of selection more rigidly and to give preference, within the greater volume of research problems, to one or another group of papers.

*SOIL SCIENCE* is to be devoted to problems in soil physics, soil chemistry and soil biology. Papers dealing with problems in plant physiology, agronomy, bacteriology or geology will be accepted only when they may contribute directly to our knowledge of soil fertility. It should not be assumed, however, that the field as outlined is at all a narrow one. The study of the mineral and organic constituents of soils, the study of soil gases, the study of soil water as a solvent of soil material, the study of soil colloids, the study of commercial plant foods and of their transformation in soils, all deal with questions as numerous as they are interesting. Added to these are the phenomena that concern soil micro-organisms, viz., bacteria, molds, protozoa and algae. Recent investigations in the domain of soil biology have shown us that this field is indeed a large one. If nothing else, these investigations have pointed out to us new modes of attack and have brought us appreciably nearer to a firmer grasp of the fundamental facts of soil fertility. It is the Editor's hope that *SOIL SCIENCE* will in some measure help to coordinate both methods and facts in soil research, and that the Consulting Editors, who so generously consented to lend their moral support to the new enterprise, will be amply repaid in the knowledge that they have made still another contribution to the progress of their chosen work.

THE EDITOR.

# A DETAILED STUDY OF EFFECTS OF CLIMATE ON IMPORTANT PROPERTIES OF SOILS.\*

BY C. B. LIPMAN AND D. D. WAYNICK.

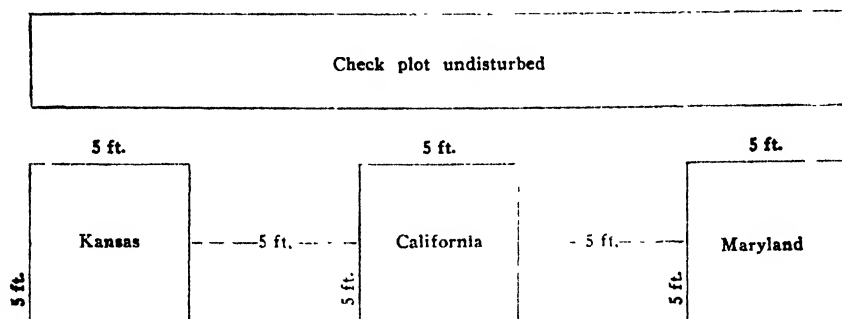
We owe to Hilgard more than to any other one investigator our prevalent conceptions with respect to the extremely important rôle played by climate as a determinant in the formation of soil types and, in a general way, of the productivity of the latter. Hilgard's admirable contributions to this subject, however, though based on a comprehensive survey of soil conditions the world over have dealt, in the main, with the causes underlying the physical and chemical constitution of soils as affected by climate. Even so far as those soil characteristics are concerned, the deductions and observations referred to, dealt with only some conditions as found at the time when the study was made, and particularly with regard to soil formation. They deal with changes wrought by the elements in agricultural soils themselves in periods of time measured secularly or millennially, rather than by intervals approximating the decade. There is as, a result, no information extant which may be employed to illuminate the subject of the effects of climatic changes, large or small, on the properties, in the broad sense of the word, of soils. It is to the task involved in such a study and in particular to changes wrought by climate in relatively brief periods of time in the same soil types that the authors addressed themselves. In planning the mode of attack in the case of such a problem, it occurred to the senior author that there was available a set of experimental soil plots which should lend themselves admirably to the purpose in view. These soil plots belong to the tri-state soil exchange experiment established for studies on climatic and soil effects on the gluten content, and on the composition, in general, of wheat. The latter experiment, which was initiated in 1908 is conducted under the joint auspices of the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture and the Departments of Agronomy respectively of the Maryland, the Kansas and the California Agricultural Experiment Stations. Detailed descriptions of the experiment are to be found elsewhere<sup>8</sup> and need receive no further consideration here. It suffices for a clear comprehension of our experiments merely to state that a soil block, five feet square and three feet deep, from the fields of each of the three state experiment stations above named, was moved to the two other experiment stations and placed in position as nearly as possible in the original order of layers. A similar block of soil was dug up and replaced in its position at every station. Strips five feet wide of untouched field soil surrounded the board frame of every plot which was placed in position. It was then possible to

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\*From the Laboratory of Soil Chemistry and Bacteriology, University of California.



study at Maryland, at Kansas and at California four soil blocks as follows: first, natural field soil *undisturbed*; second, natural field soil *disturbed* and replaced; third and fourth, soil blocks obtained respectively from each of the other two stations. The following diagram indicates the arrangement of these experimental soil blocks at the University Farm, Davis, California, and may typify the others as well.



From these plots soil samples for our experiments representing an average of each foot in depth were obtained from every station, except that through oversight no samples from the undisturbed soil at *Kansas* and at *Maryland* were obtained. The writers avail themselves of the opportunity offered in this connection to express their sense of obligation to Mr. M. A. Carleton, United States Department of Agriculture, Mr. Nickolas Schmitz, Maryland Experiment Station, Mr. F. A. Kiene, Jr., Kansas Experiment Station, and Messrs. J. W. Gilmore and B. A. Madison, of the California Experiment Station, who were instrumental in obtaining for them the soil samples from the several stations. It will be noted that a study of the samples of soil collected as above could not reveal the precise change undergone in seven years by any one soil when moved to any other station, because every one of the three soil types must have changed in its natural field position to some extent during that period. Nevertheless, such change it is only reasonable to assume, must be a relatively slight one as compared with that suffered by the same soil when removed and placed under totally different climatic conditions. Our investigations in any event can not be seriously affected by the consideration just discussed since it is our chief aim to show how any given soil in its natural location compares, after seven years, with itself under foreign conditions. Thus, for example, if the California soil at Manhattan, Kansas, or that at College Park, Maryland, now exhibits characteristics different from that now at Davis, California, the difference must be almost if not entirely referable to effects of climate. It is of course unfortunate that no detailed studies like ours, except the brief chemical studies discussed below, were made at the initiation of the soil exchange

experiment so that the data obtained therefrom might be used for comparison with our data. Finally, it must be repeated again at the risk of making this statement too prolix that it is possible to compare a given soil as it now exists at one station, at which it naturally belongs, with similar soil removed seven years ago and maintained during that period under other climatic conditions. The only permissible or possible comparison which would reveal the changes in any given soil in its natural position in a period of seven years is the one below made on the basis of its hydrochloric acid soluble constituents.

This is so because it is the only kind of study which was carried out in 1908 on the soils concerned. Even such a comparison is not without its serious drawbacks, since the chemical analysis made in 1908, or soon thereafter, was carried out by a different analyst and because the method employed was not as detailed nor quite the same as ours.

#### THE NATURE AND METHOD OF THE INVESTIGATION.

For the purpose of obtaining, as nearly as possible, a complete picture of the changes occurring in a soil when it is removed from one station to another with different climatic conditions as above explained, it was decided to study the important physical, chemical and bacteriological characteristics of the soil samples which were collected. The authors therefore determined to carry out the following studies, which may be supplemented with others later. Studies of the hygroscopic coefficient, the moisture equivalent, the wilting point, and changes in color and colloidal nature were among the physical studies made. Among chemical studies complete chemical analyses were made in accordance with the Official method, also humus and humus nitrogen determinations, total nitrogen and soil water-extract studies. Among bacteriological studies were: counts on albumen agar, ammonifying power for dried blood, nitrifying power for the soils own nitrogen, for dried blood, for cottonseed meal, for sulfate of ammonia, nitrogen fixing power in mannite solution, and qualitative tests for cellulose destruction by a method devised by the senior author. The results obtained in every class of studies are given in detail below, together with detailed descriptions of methods employed as deemed necessary.

#### DESCRIPTION OF SOILS.

The soils used in the experiments noted were described by Shaw and Walters <sup>8</sup> as follows: the California soil as "Sacramento silt loam," the Kansas soil as a "dark heavy loam" and the Maryland soil as a "light yellow clay." These terms are of little significance of course, especially since the soils have undergone so much change and such different changes at different places. So far as one of us knew them however, in the early part of the experiment, the California and Kansas soils seemed deserving

of names indicating a heavier and more tenacious condition than that indicated in the names above, and the Maryland soil of a designation indicating a much lighter condition than that of a clay soil.

### THE PHYSICAL STUDIES.

*The Hygroscopic Coefficient.* The method employed in the determination of the hygroscopic coefficient was that recommended by Hilgard, and the apparatus employed was devised by one of the authors and described elsewhere.<sup>4</sup> The determination both of the hygroscopic coefficient and the moisture equivalent were carried out by Mr. Donald E. Martin. The apparatus used for the moisture equivalent determinations is owned by the Division of Soil Technology, of the College of Agriculture of the University of California. We therefore take pleasure in expressing to Mr. C. F. Shaw, who allowed the use of his apparatus, and to Mr. Martin, who made the determinations, our appreciation of their cooperation. Table I gives the results of the hygroscopic moisture determinations.

TABLE I  
HYGROSCOPIC COEFFICIENTS.

Depth.	California Soil.			Kansas Soil.			Maryland Soil.		
	In Cal.	In Kan.	In Md.	In Cal.	In Kan.	In Md.	In Cal.	In Kan.	In Md.
1st Foot. ..	% 8.55	% 8.29	% 6.68	% 12.12	% 10.74	% 11.00	% 5.97	% 5.15	% 4.69
2nd Foot. ....	8.67	7.69	8.44	12.42	12.38	11.68	6.82	5.82	7.66
3rd Foot. ....	8.98	8.68	9.04	11.28	10.54	11.18	8.87	6.75	9.23

"Undisturbed" California Soil: 1st ft. 8.86; 2nd ft. 8.79; 3rd ft. 9.27

The results obtained with the hygroscopic coefficient determinations are not very striking. In general, it would appear that the California soil decreases in power to absorb hygroscopic moisture when placed either under Kansas or under Maryland conditions, but more particularly under the latter. In other words, the hygroscopic coefficient of the California soil is highest on its "native heath," as it were. In keeping with this general condition appears to be the behavior of the Kansas soil which when placed under California conditions increases in hygroscopicity in all three feet in depth. When placed under Maryland conditions, however, the Kansas soil behaves erratically and shows increase in hygroscopicity in the first and third foot in depth and a decrease in that respect in the second foot. In the case of the Maryland soil, the results are very irregular. Though the tendency seems to be for the Maryland soil to take up more hygroscopic moisture in California than in Kansas and in the surface foot more in Kansas than in Maryland, the soil in its natural location behaves otherwise below the surface. In general, however, the Maryland soil manifests a tendency in the same direction as the other

two, namely, to increase in hygroscopic power under California conditions. One would naturally expect that subjection of a soil to increased leaching would result in the production of more clay in it and hence render it more absorptive for hygroscopic moisture. The opposite, however, seems to be true. It seems that the explanation therefore would have to be as discussed more in detail below, that under arid conditions less clay and more aggregates being formed, the degree of packing is decreased and the total surface for hygroscopic moisture absorption is increased.

#### THE MOISTURE EQUIVALENT

That the direction taken by the data given for the hygroscopic moisture coefficients of the soils in question is not purely accidental, is indicated rather emphatically by the results obtained in determining the moisture equivalents of the same soils (Table II). It will be noted again that the California soil at California is superior to the same soil at Kansas and at Maryland. In other words, it has a greater power there to retain moisture. Likewise, the Kansas soil throughout the three-foot depth retains about 3 per cent more moisture at California than at Kansas, but it also gains slightly in power to retain moisture through its establishment at Maryland. The figures for the Maryland soil are again irregular. In spite of their irregularity, however, there can be little doubt of the marked increase in power to retain moisture acquired by the Maryland soil in the seven years of its establishment at Kansas, and though it does not gain as much in that respect at California, such gain is none the less marked enough to allow of little doubt of its existence.

TABLE II.  
MOISTURE EQUIVALENTS

Depth.	California Soil.			Kansas Soil			Maryland Soil.		
	In Cal.	In Kan.	In Md.	In Cal.	In Kan.	In Md.	In Cal.	In Kan.	In Md.
	%.	%	%	%	%	%	%	%	%
1st Foot.....	24.09	22.32	22.67	32.61	29.63	29.80	23.62	23.67	21.92
2nd Foot.....	22.81	22.20	20.32	33.33	30.78	31.14	24.26	26.02	19.37
3rd Foot.....	24.02	24.24	23.53	30.21	27.57	29.40	29.17	29.16	27.38

We have again, therefore and very much more markedly than before indirect evidence of increase in total soil surface which attends the placement for seven years of a soil under drier conditions than those under which it naturally belongs. This, it appears to us again, is contrary to what one would expect on a *priori* consideration, but is doubtless to be explained by the fact that, in general, drier climates encourage the formation of more aggregates in soils and hence permit of a looser structure and of a smaller weight of material in a given volume. Therefore, when soils are compared by equal weights it follows that more volume, hence more internal soil surface, would accompany the looser soil structure and that would result in a higher moisture equivalent.

## THE WILTING POINT.

The table below which gives the wilting points is merely interesting for purposes of a more complete view of the properties of the soils here studied. It is not of great significance, or at least is not deserving of much discussion since the values therein indicated are obtained by computation from the moisture equivalent in accordance with the formula of Briggs and Shantz.<sup>1</sup>

TABLE III.  
WILTING POINTS.

Depth.	California Soil.			Kansas Soil.			Maryland Soil.		
	In Cal.	In Kan.	In Md.	In Cal.	In Kan.	In Md.	In Cal.	In Kan.	In Md.
	%	%	%	%	%	%	%	%	%
1st Foot. ....	13.09	12.13	12.32	17.72	16.10	16.19	12.83	12.86	11.91
2nd Foot. ....	12.39	12.06	11.04	18.11	16.72	16.90	13.18	14.14	10.52
3rd Foot. ....	13.05	13.17	12.78	16.41	14.98	15.98	15.85	15.84	14.88

OTHER OBSERVATIONS ON THE PHYSICAL CONDITION OF THE SOILS  
STUDIED.

*Color*

Very marked changes take place in the color of a given soil when moved from one station to another, even in the short space of seven years. Thus the California soil changes from a distinct red brown color at California to a darker red brown at Kansas and to a dirty gray at Maryland. The three soils which seven years ago were exactly the same now show absolutely no similarity in color and as shown below very little similarity in many respects. Similarly, the Maryland soil changes from a light brown color in the surface foot at Maryland, to a dark brown in the corresponding depth at Kansas, and to a reddish brown color at California. Likewise, the Kansas soil changes in color from a deep black brown in the surface soil at Kansas to a light red brown at California and to a lighter ashen gray-brown at Maryland. These comparisons are based only on the surface soils in all cases, but the differences are so great as to mislead anyone unacquainted with these soils into thinking that any one type at the three stations is really not the same soil but three totally different soils unrelated to each other.

*Colloidal Content and Volume of Soils*

On this part of the soil studies, only preliminary observations have been completed. It appears thus far, that at their respective natural positions, the Kansas soil has the most volume per given weight, the California soil is second in that respect, and the Maryland soil last, but the latter two do not differ very markedly. When any one of these is placed elsewhere, however, great changes appear to take place in the volume of a given weight thereof even in seven years. The general tendency in that

respect, however, has not been fully enough studied to receive consideration here.

In respect to the colloidal fraction of a given weight of these soils when diffused in a large volume of water, great changes seem to be induced through climatic effects. Thus, for example, the Kansas soil at California shows much colloidal matter after 24 hours of suspension in the first foot, hardly any in the second foot, and none in the third foot. The same soil at Maryland shows a small amount of colloidal matter in suspension in the first foot, very much in the second foot, and almost none in the third foot. Again at Kansas the colloidal matter is not very marked at any depth, but much more so in the first and second foot than in the third. Similarly, as will be shown in a more detailed account to appear later, the other soils at different stations behave differently with respect to the colloidal matter in them which is capable of being suspended.

#### BACTERIOLOGICAL STUDIES.

##### *Bacterial Counts.*

Counts were made on all of the soil samples immediately after they were received. The medium employed was Brown's albumen agar. The figures obtained after the third count were taken as final and the numbers calculated for the three dilutions employed were averaged to yield the data submitted in Table IV which follows:

TABLE IV.  
BACTERIAL COUNTS

<i>California Soil.</i>						
	At California. No. per gram soil.		At Kansas. No. per gram soil.		At Maryland. No. per gram soil.	
	Bacteria.	Moulds.	Bacteria.	Moulds.	Bacteria.	Moulds.
<i>Series A.</i>						
1st Foot.....	1,820,000	14,000				
2nd Foot.....	169,333	20,000				
3rd Foot.....	108,666	20,000				
<i>Series B.</i>						
1st Foot.....	813,333	24,000	5,066,666	8,000	5,466,666	6,000
2nd Foot.....	414,666	4,000	2,140,000	8,000	926,666	2,000
3rd Foot.....	1,940,000	8,000	933,000	20,000	1,546,666	14,000
<i>Kansas Soil.</i>						
1st Foot.....	1,506,666	4,000	5,166,666	10,000	1,370,000	4,000
2nd Foot.....	1,680,000	12,000	6,100,000	None	610,000	8,000
3rd Foot.....	1,546,666	8,000	3,050,000	None	566,666	4,000
<i>Maryland Soil.</i>						
1st Foot.....	6,140,000	340,000	880,000	8,000	546,000	4,000
2nd Foot.....	800,000	200,000	210,000	6,000	400,000	2,000
3rd Foot.....	2,000	46,000	190,000	6,000	600,000	12,000

It is clear from the data in the foregoing table that even if allowance is made for the very serious errors which creep into work on bacterial counts, there is still evidence of the marked effect of climate on the number of bacteria in the several soils which will develop colonies on albumen agar. The data may be summarized as follows: The California soil in

its untouched field condition contains more bacteria in the surface foot than the same soil which has been dug up and replaced in its original position. In the second and third foot, however, and particularly in the latter, there are many more bacteria in the disturbed soil. Removal to, and retention for seven years of the California soil at Kansas and at Maryland have been instrumental in multiplying the bacterial numbers six to eight-fold in the surface foot of soil, the larger number being found in the soil at Maryland. In the second foot, however, the California soil at Kansas contains twice as many bacteria as the same soil at Maryland, but conditions are again reversed in the third foot.

Just as the California soil has its bacterial numbers enormously increased after remaining for seven years either at Kansas or at Maryland, the Kansas soil has its numbers reduced in about the same degree under similar circumstances as to time when placed either in California or in Maryland. The reduction in numbers is only slightly greater at Maryland than at California in the surface foot of soil, but is very markedly greater in both the second and the third foot of soil.

Again the Maryland soil gives unexpected results. The bacterial numbers in it increase to the greatest extent noted in all the data above submitted when it is placed and remains for some years in California. To be sure, this increase is only very marked in the first foot of soil, but it is very marked indeed. The number is only doubled in the second foot and a very striking decrease, hard to explain, occurs in the third foot. When the Maryland soil is placed at Kansas it also shows an increase in numbers in the first foot of soil, but the increase is by no means of the magnitude noted for the same soil at the California station. In the subsoil at Kansas, the Maryland soil suffers a marked decrease in numbers.

In general, therefore, it appears that the bacterial numbers in the California soil when placed at Kansas and at Maryland for the period of years noted become multiplied by four or five. Second, the bacterial numbers of the Kansas soil when placed as described at California or at Maryland become divided by four or five. Third, the bacterial numbers in the Maryland soil when placed under the conditions and time named at California and at Kansas become multiplied by ten in the surface foot at the former place, and approximately doubled in the surface soil at the latter station. In alphabetical order, therefore, the three different soils at California are approximately to one another with respect to bacterial numbers as 3 to 5 to 7. At Kansas they are about as 8 to 14 to 1, and at Maryland they are as 8 to  $2\frac{1}{2}$  to  $1\frac{1}{2}$ , all figures being based on numbers of millions of bacteria in 3 gm. of soil representing three feet in depth.

The figures given for the number of moulds in the several soils may scarcely be regarded as significant with one exception, namely, the number of moulds in the Maryland soil at California. The cause for this sudden increase in the number of moulds under the circumstances noted

is probably incapable of being expressed in simple terms. It appears, however, that the increased aeration experienced by the Maryland soil at California may have been an important factor in developing inert fungal spores contained in it in large numbers.

#### THE AMMONIFICATION STUDIES.

The method employed in the ammonification work was the one in use by most soil bacteriologists to-day and consisted in mixing 50 gm. of soil with 2 per cent of dried blood, adding the necessary water and incubating for one week. The ammonia was distilled off in the usual way and titrated. Owing to the small amount of soil available no other forms of organic nitrogen were tested for ammonifiability in these soils.

TABLE V.  
AMMONIFICATION OF DRIED BLOOD NITROGEN.

California Soil.									
	In California.			In Kansas.			In Maryland.		
	c. c. acid	Mg. N ammoni- fied.	% N ammoni- fied.	c. c. acid	Mg. N ammoni- fied.	% N ammoni- fied.	c. c. acid	Mg. N ammoni- fied.	% N ammoni- fied.
<i>Undisturbed Check Soil.</i>									
1st Foot. . .	26.5	37 10	30.16						
2nd Foot. . .	28.2	39 48	32.09						
3rd Foot . .	14.4	20 16	16 39						
<i>Disturbed Soil.</i>									
1st Foot. . .	28.1	39 34	31.98	38 9	54.46	44.27	27 9	39 06	31.75
2nd Foot. . .	18.8	26.32	21 39	23 0	32 20	26.17	22.1	30.94	25.15
3rd Foot . .	16 2	22 68	18 43	17 3	24 22	19.68	18 3	25.62	20.82
<i>Kansas Soil.</i>									
1st Foot. . . .	22.7	31.78	25 83	31.1	43 54	35 39	30 5	42.70	34 71
2nd Foot. . . .	23.3	32.62	26 52	28.2	39.48	32 09	24.8	34.72	28.22
3rd Foot. . . .	28.6	40 04	32.55	23.2	32 48	26.40	20.6	28.84	23.44
<i>Maryland Soil.</i>									
1st Foot. . . .	27.2	38.08	30 95	34 3	48.02	39.04	29 7	41.58	33.80
2nd Foot. . . .	22 4	31.36	25.49	21.5	30.10	24 47	20 5	28 70	23.33
3rd Foot. . . .	20.2	28 28	22.99	19.2	26.88	21 85	19.1	26.74	21.74

A study of Table V, which sets forth the ammonification data which the authors obtained, reveals most strikingly of all the investigations thus far reported in this paper how significant a rôle is played by climate as a determinant of soil flora. We see first on studying the data more closely that by merely disturbing the California soil at its own natural location and replacing it no very profound changes are effected. Nevertheless such procedure does slightly increase the ammonifying power of the surface foot of soil and rather markedly decreases it for the second foot. The third foot gains slightly in ammonia producing power by being moved and replaced. When we now consider the effects on the ammonifying power of the California soil of seven years of exposure under California, Kansas and Maryland conditions respectively, we find them to be on the whole much more profound than those heretofore discussed. Thus contrasting the ammonifying power of the California soil similarly treated at



California and Kansas we find it to be very considerably larger at the latter location—indeed to the extent of being capable of rendering about 44 as against about 31 per cent of the nitrogen in dried blood into ammonia. The same climatic effects which have been instrumental in causing such a striking change in the ammonifying power of the first foot of the California soil at Kansas have extended their influence even into the second and third feet of the same soil at the same location. Thus the second foot renders over 5 per cent more and the third foot over 1 per cent more of the nitrogen supplied in the 2 per cent of dried blood into ammonia than do similar depths of the same soil which is located at California. In the case of the California soil which is installed at Maryland, however, we find relatively little difference in ammonifying power from that characteristic of the same soil in its natural location. Moreover, such differences as have made themselves apparent can be noted only in the second and in the third foot of the soil which resembles very closely in ammonifying power the corresponding depths of the same soil at Kansas.

Considering now the Kansas soil we find it to be a vigorous ammonifying soil in all three depths at Kansas. When, however, it is allowed to remain as above indicated for seven years at California, it loses markedly in ammonifying power in both the first and second foot of soil, though it gains in that respect in the third foot. The loss in ammonifying power suffered by the first foot of soil is not quite so great as the gain in that direction accruing to the California soil when similarly placed at Kansas, but is nevertheless decidedly of the same order of magnitude. Thus about 10 per cent more of the nitrogen in dried blood is ammonified in the Kansas soil at Kansas than in that at California; about 5 per cent more is thus transformed in the second foot of the Kansas soil at its natural location than at California. When however, the Kansas soil is placed at Maryland as explained, it suffers little modification in ammonifying power, if any, in the surface foot of soil, but does lose in that respect in the second and third foot.

In the case of the Maryland soil we are permitted again to gain an insight into the superiority of Kansas climatic conditions for increasing the ammonifying power of a soil over those of California and Maryland. Thus while at its natural location the Maryland soil is slightly inferior to the Kansas soil in ammonifying power, absolutely speaking, it becomes very considerably superior to it in that respect when placed at Kansas under the conditions noted. Even in the subsoil the ammonifying power of the Maryland soil is improved by its transfer to Kansas. When, however, the Maryland soil is placed at California, like the Kansas soil at the latter place, it loses in ammonifying power in the surface, though less slightly, and only in the surface soil. In the subsoil, it gains slightly in ammonifying power.

Studying now all of the figures in Table V, in the more absolute sense we find that the highest ammonifying efficiency attained by any of the soils there described is that of the California soil at Kansas and the Maryland soil at Kansas is second, when the surface foot of soil alone is considered. When the three-foot column of soil is considered, the Kansas soil at Kansas stands first in ammonifying efficiency and the California soil at Kansas second. Therefore, when the first foot alone is considered the Kansas soil at Kansas stands third and when the three-foot column is considered, the Maryland soil stands last.

At California the California soil is the most efficient ammonifier of dried blood nitrogen in the surface foot, but the Maryland soil at the same place is a close second with the Kansas soil decidedly behind. When, however, the three-foot column is considered the Kansas soil is first at California as it is at Kansas in ammonifying power, the Maryland soil is second, and the California soil is last.

At Maryland the Kansas soil is first in ammonifying efficiency in the first foot, the Maryland soil is second and the California soil last. The differences between the soils are here less marked, however, than at other places as above discussed. When the three-foot columns at Maryland are considered by averages the Kansas soil is first in ammonifying efficiency as it is at both California and Kansas, the Maryland soil is second and the California soil is last. In this respect therefore the three soils occupy similar relative positions to each other, both in California and in Maryland, and with one change for second place are also similarly situated at Kansas.

#### THE NITRIFICATION STUDIES

The method employed in the nitrification studies is one which the senior author, in common with other investigators, has employed for several years. Briefly, it consists in studying the nitrifying power of 100 gm. of soil for a given form of nitrogen at an incubator temperature of about 28° C. during a period of about four weeks. In this case, however, the tests were more elaborate than usual, since four forms of nitrogen were employed, namely, the soil's own nitrogen (nitrogen in the untreated soil), cottonseed meal, sulfate of ammonia, and dried blood. In the first case 100 gm. of the air-dry soil were placed in a tumbler, enough water added to make as nearly as possible optimum moisture conditions, the whole thoroughly stirred, the tumbler covered with a Petri dish cover, and incubated as above indicated. In the case of the cottonseed meal the procedure differed from that just given only in that 1 per cent of cottonseed meal was thoroughly stirred into the soil in its dry condition before water was added. The dried blood cultures were similarly treated to the cottonseed meal culture, but in the case of sulfate of ammonia not 1 per cent but .2 per cent of the latter was added to the soil in solution

and the water content made up as described in the other cases. The results obtained showing the number of milligrams of nitrate nitrogen produced (net) in the four week's incubation period, together with the percentage of the total nitrogen present in the soil or the fertilizer, rendered into nitrate in every case, are set forth in Table VI which follows.

TABLE VI.  
CALIFORNIA SOIL—NITRIFICATION.  
*In California, Series A (Undisturbed Soil).*

	Soil Nitrogen.		Cottonseed Meal.		Sul. of Ammo'ia.		Dried Blood.	
	Mg. N nitri- fied.	% N nitri- fied.	Mg. N nitri- fied.	% N nitri- fied.	Mg. N nitri- fied.	% N nitri- fied.	Mg. N nitri- fied.	% N nitri- fied.
1st Foot.....	5.80	12.00	8.20	29.80	4.40	20.95	24.80	38.15
2nd Foot.....	3.83	9.11	7.83	28.46	2.83	13.49	11.83	18.20
3rd Foot.....	.95	2.61	5.35	19.44	3.55	16.95	14.85	22.84

*In California, Series B (Disturbed Soil).*

1st Foot.....	4.78	9.88	9.78	35.56	5.08	24.19	16.78	25.81
2nd Foot.....	3.90	9.60	lost	..	4.30	20.47	16.90	26.00
3rd Foot.....	2.91	8.66	5.91	21.12	5.91	28.14	15.91	24.47

*In Kansas.*

1st Foot.....	4.64	10.35	19.64	71.41	2.24	10.66	.52	.80
2nd Foot.....	3.70	10.36	12.70	46.18	1.46	6.95	1.10	1.53
3rd Foot.....	2.50	8.71	14.70	53.45	1.46	6.95	.50	.77

*In Maryland.*

1st Foot.....	4.79	10.36	12.79	46.50	3.29	15.66	.79	1.21
2nd Foot.....	2.45	7.44	14.85	54.00	2.25	10.71	.15	.23
3rd Foot.....	2.72	8.44	13.92	50.61	1.92	9.14	.02	Trace

# KANSAS SOIL—NITRIFICATION.

*In California.*

1st Foot.....	4.33	6.72	13.93	50.65	19.93	94.90	33.93	36.81
2nd Foot.....	3.05	6.91	13.85	50.36	21.85	100.40	20.85	32.07
3rd Foot.....	lost	.....	19.70	71.63	21.70	100.30	8.70	11.84

*In Kansas.*

1st Foot.....	5.70	8.06	24.70	89.81	3.80	18.09	20.70	31.84
2nd Foot.....	4.20	8.95	21.60	78.54	5.60	26.66	4.10	6.30
3rd Foot.....	2.15	7.48	22.65	82.36	14.65	69.76	2.05	3.15

*In Maryland.*

1st Foot.....	5.86	9.73	24.86	90.40	3.86	18.38	3.66	5.58
2nd Foot.....	5.29	12.18	22.89	83.23	5.39	25.66	.89	1.36
3rd Foot.....	3.93	11.22	19.93	72.47	4.43	21.09	.33	.51

# MARYLAND SOIL—NITRIFICATION.

*In California.*

1st Foot.....	7.20	11.82	29.60	100.76	2.60	12.38	15.60	24.00
2nd Foot.....	.90	2.76	.65	2.36	1.90	9.04	20.90	32.15
3rd Foot.....	.90	2.79	— 20	.....	— .40	.....	1.60	2.46

*In Kansas.*

1st Foot.....	8.20	14.82	26.00	94.54	— .10	.....	4.00	6.15
2nd Foot.....	4.90	15.21	1.30	4.72	— .70	.....	— .50	.....
3rd Foot.....	2.20	6.98	— .70	.....	— .60	.....	— .60	.....

*In Maryland.*

1st Foot.....	7.78	15.65	10.78	39.20	— .22	.....	— .02	.....
2nd Foot.....	.93	3.24	.03	Trace	— .27	.....	— .17	.....
3rd Foot.....	.60	2.14	.35	1.27	— .15	.....	— .05	.....

The data submitted in the foregoing table are deeply interesting. They indicate very emphatically that climate plays a most significant rôle in determining the nitrifying powers of surface soils and of subsoils. They also show that the rôle thus played by climate may be in increasing or in diminishing a soil's nitrifying power by any given change in climate, depending upon the form and quantity of nitrogen which is being nitrified.

*The California Soil—The Soil's Own Nitrogen.*

Considering now in more detail data with reference to which the foregoing general statement is made, it is found first that while the disturbance and replacement of the California soil at California has caused a loss in the nitrifying power for its own nitrogen of the surface foot, it has caused a gain in that respect in the third foot; and the net result owing to the soil's disturbance is a considerable gain in the amount of nitrogen transformed into nitrate in three feet of soil. But even such improvement which probably results from increased aeration of the California soil at its own location is not as efficient in the respect noted as the establishment of the California soil at Kansas. At the latter place the California soil not only improves in nitrifying power in the sub-soil, but also surpasses its own record in the two surface feet of soil. At Maryland, however, while the nitrifying power of the surface foot of California soil is equal to that at Kansas, the second foot is considerably inferior to that of the same soil at the latter place. When, therefore, the three-foot columns of the California soil at the three stations are compared, that at Kansas is first, that at California second, and that at Maryland third in nitrifying power for the soil's own nitrogen. When, instead of comparing these soils on the basis of the percentage of nitrogen transformed into nitrate, we compare them on the basis of the absolute amounts of nitrate produced, the California soil at California stands first, that at Kansas is second, and that of Maryland is again third.

*Cottonseed Meal Nitrogen.*

The improvement wrought by the disturbance and hence aeration of the California soil at California, so far as its nitrifying power for its own nitrogen is concerned, is only intensified when its power to transform cottonseed meal nitrogen into nitrate is in view. This is true on the basis of the absolute amounts of nitrate produced as well as on that of the percentage of the total nitrogen in the cottonseed meal which is rendered into nitrate. Distinct as it appears to us to be, however, this degree of improvement in the direction noted in the California soil is greatly surpassed when compared with the improvement, both absolute and relative, which is induced by the establishment of the California soil at Kansas for the period here under consideration. The nitrifying power for cottonseed meal nitrogen of the California soil is so greatly improved

at Kansas that it transforms over 71 per cent of the total nitrogen in the cottonseed meal into nitrate in the surface foot of soil. This is among the most efficient transformations of cottonseed meal nitrogen noted by the writers in their nitrification work. When placed at Maryland, the California soil again gains very markedly in nitrifying power for cottonseed meal nitrogen, though not so markedly as it does at Kansas.

On the basis therefore of its power to render cottonseed meal nitrogen into nitrate, the California soil is to be appraised as follows: 1st at Kansas, 2nd at Maryland, 3rd at California.

#### *Sulfate of Ammonia Nitrogen.*

The disturbance of the California soil at California is instrumental even more markedly than in the case of cottonseed meal nitrogen in increasing its power to produce nitrate from sulfate of ammonia nitrogen in the third foot of soil. In striking contrast with the case of cottonseed meal nitrogen, however, the California soil when established at Kansas or at Maryland so far from gaining enormously in power to nitrify sulfate of ammonia nitrogen actually loses markedly in that respect at both places and particularly enough more markedly at Kansas than at Maryland. In other words, when sulfate of ammonia nitrogen is involved the nitrifying power of the same soil at the three different stations is in the following order the most efficient being placed first: 1st California soil at California, 2nd at Maryland, 3rd at Kansas.

#### *Dried Blood Nitrogen.*

When dried blood nitrogen is the form employed to test the soil's nitrifying power enormous changes, perhaps the most striking of all, are indicated by the removal of the soil to Kansas and to Maryland. Such changes are in the opposite direction to that noted in the case of the cottonseed meal nitrogen. Thus the California soil which when disturbed and aerated at California changes in nitrifying power for dried blood nitrogen much as it does for its own nitrogen there, still shows a good nitrifying power in either case. When, however, it is established at Kansas or at Maryland it almost loses in its entirety its power to nitrify dried blood nitrogen when the latter is employed in the quantity noted for the nitrification tests.

### NITRIFICATION STUDIES—KANSAS SOIL.

#### *The Soil's Own Nitrogen.*

When placed at California the Kansas soil loses decidedly in nitrifying power for its own nitrogen, but when placed at Maryland it gains in that respect even more decidedly. This is just as true for the whole three-foot soil column as it is for any given sample. That the first should occur is intelligible to us and reasonably explicable on the basis

of changes in the organic matter content of the soil transported from Kansas to California. It is not clear how the latter occurs, however, unless we assume, which perhaps is true, that more organic matter has accumulated in the Kansas soil at Maryland than at its natural location. When, therefore, the California soil in three-foot columns is compared with the Kansas soil it is found that the former loses somewhat on the basis of absolute amounts of nitrates produced by being moved either to Kansas or to Maryland. The latter however, is improved markedly by its sojourn in Maryland and loses decidedly at California. In other words, climate does not seem to operate in the same direction, since the climate under which the California soil is most efficient as a nitrifier of its own nitrogen is the one under which in seven years the Kansas soil is rendered least efficient as among the three locations under which it was tested. Again, the California soil is least efficient in the direction noted under Maryland conditions, yet it appears that, of the three studied, the latter are the most propitious for the Kansas soil.

#### *Cottonseed Meal Nitrogen.*

Cottonseed meal nitrogen seems to be affected in a manner similar to that of the soil's own nitrogen in the Kansas soil, but differences in degree are decidedly apparent. Thus while the Kansas soil when placed at Maryland is perhaps slightly superior in nitrifying power to itself when allowed to remain in Kansas, such superiority is very slight. On the other hand, as a nitrifier of cottonseed meal nitrogen, the Kansas soil at California is decidedly inferior to that at Maryland or at Kansas. Both the absolute and relative amounts of nitrates produced from cottonseed meal nitrogen are especially worthy of note as indicating the high availability of the latter in that soil.

#### *Sulfate of Ammonia Nitrogen.*

The most noteworthy and only really remarkable figures obtained for the nitrification of sulfate of ammonia nitrogen were obtained with the Kansas soil at California. The absolute amounts of nitrates produced were so large as to indicate, if the soil nitrogen were not involved, an almost complete transformation of the ammonia nitrogen added into nitrate in the whole three-foot columns. The figures given there as well as in one or two other places in the table which show more than 100 per cent availability of nitrogen are of course to be explained on the basis of a nitrification of the soil nitrogen plus the added ammonia nitrogen. Since such a sum is usually an algebraic one, it may readily be inferred that even more nitrates may have been produced in the cultures concerned and again lost sometime during the incubation period. Briefly, therefore, as the table indicates and just contrary to the cases of the soil's nitrogen and that of the cottonseed meal, the Kansas soil gains very markedly in nitrifying power for sulfate of ammonia nitrogen by being

placed at California. In the third foot, however, while the Kansas soil at California is still far superior to that at Kansas in the direction noted, the latter shows great activity. At Maryland, however, the Kansas soil is even a relatively less efficient nitrifier of sulfate of ammonia nitrogen than at Kansas, and in the third foot decidedly less so.

#### *Dried Blood Nitrogen.*

In respect to dried blood nitrogen, the Kansas soil, as to location, acts in a manner similar to its action with sulfate of ammonia nitrogen. In other words, it nitrifies blood nitrogen more efficiently at California than it does at its own natural location in the whole three-foot column, but particularly in the subsoil. On the other hand, when it is moved to Maryland it loses, even more strikingly than in the case of sulfate of ammonia nitrogen, its power to produce nitrates from dried blood nitrogen. At the latter place, indeed, its nitrifying power for dried blood nitrogen is feeble in the surface foot of soil and almost nil below.

#### NITRIFICATION STUDIES—MARYLAND SOIL.

##### *The Soil's Own Nitrogen.*

The table above submitted indicates that there can be no question about the profound effect of the Kansas climate on the nitrifying power of the Maryland soil for its own nitrogen, particularly so far as the subsoil is concerned. Thus of the total nitrogen found in the three-foot column of the Maryland soil at Kansas, over 37 per cent is nitrified, whereas of the total nitrogen in the same soil at Maryland (its natural location) only about 21 per cent is nitrified. At California, on the other hand, the nitrifying power of the Maryland soil remains practically unchanged, from its condition at Maryland. The surface soil does seem to lose in nitrifying power slightly, but no conclusions may be drawn from that fact.

It is to be particularly remarked here, however, that the nitrogen of the Maryland soil is far more readily nitrified than that of the Kansas and California soils, even though we are obliged to draw this conclusion from the data for the surface foot of soil only.

##### *Cottonseed Meal Nitrogen.*

Unlike the Kansas and the California soils, the Maryland soil does not show parallelism in general direction between its power to nitrify cottonseed meal nitrogen and its own nitrogen. Thus at California the Maryland soil nitrifies cotton seed meal nitrogen more efficiently than at Kansas, and does nearly three times as well at the first mentioned place as it does at its natural location in Maryland. It is worthy of note also that while the soil's own nitrogen in the Maryland soil at Kansas is readily nitrified in the subsoil, the same soil from the same place shows only a feeble nitrifying power for cottonseed meal nitrogen, despite the fact that

the latter nitrifies very much better than the soil nitrogen in the first foot of soil. It is further to be remarked that the maximum absolute amount of nitrate obtained in any of the cultures reported in the foregoing table was obtained in the Maryland soil at California, and that at Kansas was a close second. These records are, to be sure, made only in the first foot of soil. We may also add that the percentage of total nitrogen transformed in the two record cultures referred to are above 100 per cent and above 94 per cent respectively. These figures are of course somewhat exaggerated since they represent nitrates produced from the soil nitrogen as well as that from cottonseed meal nitrogen, but the latter alone was used in the calculation above made. The reason as explained above for not subtracting the amount of nitrate produced from the soil nitrogen from the total obtained in the cottonseed meal cultures is that the latter amount represents the algebraic and not the arithmetical sum of nitrification activities in those cultures. For the first time among all the cultures discussed in the nitrification studies we are confronted by a subsoil in the Maryland soil at all stations which is either very feeble in nitrifying power or possesses none at all for cottonseed meal nitrogen.

#### *Sulfate of Ammonia Nitrogen.*

Among the most striking results obtained with the Maryland soil are the nitrification figures for sulfate of ammonia nitrogen. Not only is the Maryland soil at both Maryland and Kansas incapable of nitrifying sulfate of ammonia nitrogen in the quantity used, but the addition of sulfate of ammonia actually causes a loss of the nitrate nitrogen initially contained in the soil. At California, on the other hand, the Maryland soil has evidently become so changed in seven years as to be capable of nitrifying sulfate of ammonia nitrogen in both the first and second feet, though losing nitrates as in the other cases, in the third foot. The absolute and relative magnitude of the transformation of the ammonia nitrogen in question into nitrate in the Maryland soil at California is approximately that of the California soil at Kansas, and considering the first two feet of soil alone is probably slightly better. The acid condition of the Maryland soil is probably chiefly responsible for its behavior with respect to sulfate of ammonia.

#### *Dried Blood Nitrogen.*

At Kansas and at its natural location the Maryland soil behaves with respect to dried blood nitrogen very much as it does toward sulfate of ammonia nitrogen, except that it does nitrify the former to some extent in soil from the first foot at Kansas. Likewise, at California there is a similar behavior, in kind between the Maryland soil with blood nitrogen and ammonia nitrogen. In degree, however, there is a marked difference, the blood nitrogen being much more vigorously nitrified in the first two



feet of soil than the sulfate of ammonia nitrogen and even in the third foot of soil it is appreciably nitrified. So far as dried blood nitrogen is concerned, therefore, the Maryland soil is clearly improved in the first foot in nitrifying power by being placed at Kansas, and very markedly improved in the whole soil column in that respect by being placed for the same period of seven years at California. The possible causes of the non-nitrification of blood nitrogen in the Maryland soil at some places and its ready nitrification in other places are discussed elsewhere in this paper.

#### MANNITE SOLUTION AND NITROGEN FIXATION STUDIES.

Owing to the small quantity of soil available we were compelled to use the mannite solution method for determining the nitrogen fixing powers of the soil. It is of course regrettable that the soil itself could not be employed as a medium, as was the case in other tests above noted. The data obtained, however, are very interesting and the method employed, while defective in some respects, is valuable in that the *Azotobacter* and other bacterial forms involved could be studied more in detail thereby, as the descriptions below indicate. The cultures which were incubated for two weeks were prepared by inoculating 5 gm. of the soil to be tested into 50 c.c. mannite solution. The nitrogen determinations were made by the modified Gunning method in use in this laboratory and a description of it may be found elsewhere.<sup>3</sup> The results of the observations made after one and after two weeks' incubation on every culture were as follows:

#### CALIFORNIA SOIL.

##### "Undisturbed."

- First Foot.* Butyric odor, very few *azotobacter* cells visible under microscope. Gas formation marked. Membrane slow to develop. Same after two weeks.
- Second Foot.* Slightly esteric odor, good *azotobacter* development. Gas formation marked. Membrane thin, mucilaginous. Same after two weeks.
- Third Foot.* Odor more markedly esteric than that of second foot. A few *azotobacter* cells. Membrane slight. Gas formation marked. Same after two weeks.

##### *Disturbed.*

- First Foot.* Fairly strong esteric odor, also slightly butyric. A few *azotobacter* cells. Membrane slow to develop. Gas formation marked. Same after two weeks, but less cells.
- Second Foot.* Esteric odor. Few *azotobacter* cells. Slight membrane. Gas formation marked. Same after two weeks.
- Third Foot.* Odor same as above. Membrane very heavy and mucilaginous. *Azotobacter* numerous. Gas production marked.

**At Kansas.**

- First Foot.** Slightly fetid, also esteric odor. Heavy membrane, granular. Little, if any, gas formation. *A. chroococcum* very plentiful. Pigment marked, brown-gray; same after two weeks, but deeper color.
- Second Foot.** Odor same as above. Membrane not granular but continuous, dark, silver gray pigment. Very little *A. chroococcum*, if any, visible. Other azotobacter forms. Gas formation same as above. Same after two weeks.
- Third Foot.** Odor same as above. Membrane same as first foot, but pigment light silver gray. *A. chroococcum* as in first foot. Gas formation same. Same after two weeks.

**At Maryland.**

- First Foot.** Fetid odor almost masks strong butyric odor. Heavy membrane. Pigment brownish gray. Very little gas formation. *A. chroococcum* not as numerous as in same soil at Kansas. Other azotobacter forms numerous. Same after two weeks, except pigment dark brown to black.
- Second Foot.** Same odor as first foot. Membrane same but pigment silver gray. Almost no gas. Azotobacter forms as in first foot. Same after two weeks.
- Third Foot.** Odor and membrane as in first foot. Pigment, however, is yellowish. Heavy gas formation. Azotobacter as in first foot. Same after two weeks.

## KANSAS SOIL.

**At California.**

- First Foot.** Mild esteric odor. Many azotobacter cells, but no membrane. Gas production marked. Same after two weeks.
- Second Foot.** Mild esteric odor. Very many azotobacter cells, but no membrane. Gas production marked. Same after two weeks.
- Third Foot.** Mild esteric odor. Only few azotobacter cells. No membrane. Gas production marked. Same after two weeks.

**At Kansas.**

- First Foot.** Decidedly fetid odor. Good membrane. Pigment none, but gray color. Moulds present on membrane. No gas. Same after two weeks, except odor not so strong, much more mould and blue to purple color appearing.
- Second Foot.** Strong, pleasant, esteric odor. Membrane same as in first foot. Azotobacter present in both. Color light silver gray. No pigment. No gas. No mould. Same after two weeks.
- Third Foot.** Same as second foot, but color is yellowish gray. No mould.

**At Maryland.**

- First Foot.** Fruit and nut odor. Very heavy membrane. No gas. Several species of Azotobacter appear to be present. Much mucilaginous material in membrane. Slightly dark pigment after two weeks.
- Second Foot.** Same as first foot except distinct azotobacter species appear to be present in addition. Not as much mucilaginous material in membrane. Odor weaker. After two weeks very dark pigment.
- Third Foot.** Same as first foot, but gas present and pigment more marked. After two weeks gas has disappeared and moulds appearing.

## MARYLAND SOIL.

*At California.*

- First Foot.* Strong butyric odor. Clostridium plentiful, but no Azotobacter. Membrane superimposed on gas bubbles made up of fungus mycelium. Same after two weeks.
- Second Foot.* Weak esteric odor. Clostridium present, but no Azotobacter. Membrane superimposed on gas bubbles, as in first foot. Same after two weeks.
- Third Foot.* Weak esteric odor. Clostridium found, but no Azotobacter. Heavy mould membrane. Same after two weeks.

*At Kansas.*

- First Foot.* Butyric odor. No membrane. Gas formation plentiful. No moulds. No azotobacter forms visible. After two weeks the same, except a few cells found which resemble Azotobacter.
- Second Foot.* Odor somewhat esteric, but different from any thus far noted. Less marked gas formation than above. No Azotobacter. No moulds. After two weeks odor changed to that of mercaptan. Heavy algal growth also has appeared.
- Third Foot.* Much the same throughout as second foot.

*At Maryland.*

- First Foot.* Strong butyric odor Clostridium forms plentiful. No membrane. Fair gas production. A few cells resembling Azotobacter. No pigment. No color. After two weeks a little more gas formation, but otherwise the same.
- Second Foot.* Same as first foot, except bacteria more numerous and show more activity.
- Third Foot.* Same as first foot.

## THE NITROGEN FIXATION DATA.

Before discussing the actual and probable significance of the data on the mannite solution cultures which are above set forth it may be well to consider the data obtained with the same culture as regards nitrogen fixation. The nitrogen was determined as above stated after the two weeks' incubation period and the amounts of nitrogen found in the cultures as well as that actually fixed from the atmosphere are given in Table VII which follows.

It is interesting to note by way of general correlation of the chemical data with the microscopic and macroscopic observations on the mannite solution cultures, that a parallelism exists between the magnitude of the amounts of nitrogen fixed and the presence or absence of Azotobacter. Very vigorous azotobacter membrane development is always accompanied by high nitrogen fixation. Per contra, slight membrane formation or relative scarcity of azotobacter cells is always associated with low nitrogen fixation. Nevertheless, even the total absence of azotobacter organisms as is clearly brought out above does not prevent the soil from fixing nitrogen, though the amounts fixed may be much smaller

than is the case in the presence of those organisms. This observation is in harmony with a similar one previously made by the senior author in other mannite solution studies.<sup>6</sup> Not only the presence of *Azotobacter*, but also the thickness of the membrane, and the rapidity of its development are excellent indicators of the degree of nitrogen fixation.

TABLE VII.  
NITROGEN FIXATION IN MANNITE SOLUTION.  
*California Soil (Disturbed).*

	In California.		In Kansas.		In Maryland.	
	Mg. N found	Mg. N fixed per gram mannite	Mg. N found	Mg. N fixed per gram mannite	Mg. N found	Mg. N fixed per gram mannite
1st Foot . . . . .	8.82	3.92	14.28	9.80	14.28	9.66
2nd Foot . . . . .	8.54	4.48	12.04	8.54	12.32	9.10
3rd Foot . . . . .	8.54	5.18	13.58	10.78	12.88	9.66
<i>Undisturbed</i>						
1st Foot . . . . .	8.96	4.20				
2nd Foot . . . . .	9.38	5.18				
3rd Foot . . . . .	7.84	4.20				
<i>Kansas Soil.</i>						
1st Foot . . . . .	6.58	1.14	16.94	9.94	16.66	10.64
2nd Foot . . . . .	8.40	4.06	13.16	8.54	14.98	10.64
3rd Foot . . . . .	10.50	7.28	11.90	9.10	13.02	9.52
<i>Maryland Soil.</i>						
1st Foot . . . . .	9.52	3.50	8.96	3.50	10.22	5.32
2nd Foot . . . . .	6.44	3.22	7.56	4.34	8.12	5.32
3rd Foot . . . . .	6.30	3.08	7.28	4.20	6.16	3.36

When more specifically discussed, the data submitted offer numerous points of interest. The disturbance of the California soil without any other modification reduces nitrogen fixation and *azotobacter* development in the first and second foot and increases it in the third foot. Removal of the California soil to Kansas increases the vigor of the *azotobacter* flora and especially that of *A. chroococcum* and increases the nitrogen fixation by 50 per cent of that attained by the same soil at California. Removal of the California soil to Maryland is nearly, if not quite, as efficacious a measure for invigorating the *azotobacter* flora and increasing nitrogen fixation as the removal of the soil to Kansas.

The Kansas soil at Kansas shows a vigorous *azotobacter* flora and gives evidence of vigorous nitrogen fixing power. With the exception of the third foot which does not fix as much nitrogen, the Kansas soil at Kansas is about equally good at nitrogen fixation with the California soil at Kansas. When, however, the Kansas soil is removed to California and remains there as observed, it loses its power to produce a membrane in mannite solution, the *azotobacter* flora become rather feeble and the number of cells few and nitrogen fixation power is reduced to almost nothing in the first foot, by more than 50 per cent in the second foot, and by about 20 per cent in the third foot. These data were so striking that the

cultures were repeated with new soil obtained from the same plots. Similar results were obtained and they differed only in that the first foot fixed .70 mg. instead of .14 mg. nitrogen, and in that membranes were formed in the second and third foot and nitrogen fixation was correspondingly increased there. Even in this more favorable series of cultures the fixation of nitrogen was very much lower in the Kansas soil at California than in that at Kansas. Since the second set of samples was taken at a different season the results therewith are not strictly comparable with those above submitted, and they are therefore not given here. Removal of the Kansas soil to Maryland so far from inducing deterioration and enfeeblement of the azotobacter flora as was the case in removing that soil to California, actually increases the vigor of those organisms and conduces to the fixation of more nitrogen than that accomplished by that soil at its home location. Indeed, the greatest fixation of nitrogen in any of the cultures is accomplished by the Kansas soil at Maryland when the whole three-foot column is considered. The cause for the behavior of the Kansas soil at Maryland is not easy to discover. It is probably to be found in some of the intricate relationships induced in soils by climate which will be discussed, in general, below.

The Maryland soil at Maryland has a slightly higher nitrogen fixing power than the California soil at California, despite the fact that the latter has a relatively feeble azotobacter flora and that the former probably has no azotobacter organisms. Still the difference is very slight when the whole three-foot column is considered, and the very vigorous *Clostridium* flora in the Maryland soil probably offset the activity of the feeble azotobacter flora in the California soil. However, that may be, the case under discussion is no serious objection to the correlation drawn above between nitrogen fixation and the presence of vigorous azotobacter flora since from the absolute standpoint the nitrogen fixation (in mannite solution) in both soils here discussed is decidedly low. When the Maryland soil is placed at California it appears to lose in nitrogen fixing power as does the Kansas soil when similarly placed, but not so markedly as the latter. Moreover, it develops very vigorous mould flora which does not appear to be present in the same soil at its home site. This mould flora forms a veritable thick membrane at the surface of the mannite solution which is observed nowhere else in the cultures here discussed. Whether the decrease in nitrogen fixation is caused by an enfeeblement of the *Clostridium* flora through increased aeration or is in some way connected with a loss of nitrogen through the heavy mould development is impossible at this time to state. By reference to the tables given below it will be seen that another puzzling element enters into this problem. The Maryland soil seems to gain appreciably in total nitrogen through its sojourn in California during a stated period. Whether, in connection with the

nitrogen fixation data above considered this be a causal relationship or merely one of effect remains also an open question. At Kansas the Maryland soil also loses in nitrogen fixing power but not nearly as much and it even shows an appreciable gain in the third foot. In this case the question of mould development does not enter in as observed in the description above given. On the other hand a vigorous algal development principally of Cyanophyceae is noted in the second and third foot. Since algae have been on many other occasions shown to be more or less directly connected with stimulation to non-symbiotic processes of nitrogen fixation, their presence, particularly in the third foot of Maryland soil at Kansas, may not be without significance. In general, however, and as a soil column the Maryland soil loses in nitrogen fixing power by being removed either to California or to Kansas. Since its nitrogen fixing powers are probably entirely dependent or nearly so, on *Clostridium* and other anaerobic non-symbiotic nitrogen fixing organisms, the observed facts are probably explicable on the ground of the increased aeration given the soil at both California and Kansas, which would operate towards the inhibition of the activities of the organisms mentioned.

The long period of drouth and loss of organic matter suffered by all the soils at California is probably also responsible for the partial destruction and hence enfeeblement of the nitrogen fixing flora. This might perhaps help to explain not only the facts noted in the case of the Maryland soil just discussed, but also that of the Kansas soil and the striking results obtained with it at California. On the other hand, the same idea would explain the increase in activity as regards nitrogen fixation manifested by the California soil at Kansas and at Maryland and by the Kansas soil at Maryland on grounds of differences in the amount and distribution of precipitation which are too well known to need amplification here.

It is hoped that further investigations now in progress will demonstrate whether or not the observations made above together with general observations to be made below are adequate to explain the facts set forth with reference to nitrogen fixation. Irrespective of that, however, it appears to be certain from the data thus far in hand, that the nature of the nitrogen fixing flora of a given soil and characteristics pertaining thereto, are not to be accounted for under the conditions here studied, by a mere contamination of one soil by another, in the experiment described, for which there has been given the most ample opportunity. Such may appear to be the case, for example, when the nitrogen fixation attained by the Kansas soil at Kansas and the California soil at Kansas are compared. The idea fails of substantiation, however, when we observe that the California soil at Maryland does not exhibit characteristics of the Maryland soil at Maryland, but rather those resembling the Kansas soil at Kansas. Likewise the Maryland soil at Kansas does not behave as

the Kansas soil at Kansas, but more closely resembles in behavior the California soil at California. So too, if we may be allowed another example to illustrate the point we are attempting to make, the Kansas soil at California does not at all resemble the California soil at that place in respect to nitrogen fixation, and indeed behaves unlike any other soil there, it being the only soil that in the surface foot fixes scarcely any nitrogen.

#### STUDIES ON CELLULOSE DESTRUCTION.

The method employed for comparing the cellulose destroying powers of the soils here studied was not a quantitative one, but consisted of the following procedure. Thirty grams of soil were placed in a Petri dish and smoothed off on the surface as much as possible. A little more moisture than was necessary for optimum conditions was then added after placing a disk of ashless filter paper on the soil surface. This procedure insured almost uniform contact of filter paper and soil. The dishes were then covered and incubated at 28° to 30° C. for four weeks. Most of the cellulose destruction noted was carried out in the first eight or ten days. The results obtained are set forth in the photographs which are submitted herewith in Plates I, II, III, IV, and V.

A study of the photographs brings out very strikingly the relationships obtaining between the various soils and cellulose destruction. Not only is the surface foot of the California soil more vigorous at cellulose destruction than the corresponding depth of the Kansas or the Maryland soil, but the second and third foot thereof are very vigorous in that respect, whereas, corresponding depths of the other soils have dissolved none or scarcely any portion of the filter paper. Moulds also are very much more numerous in the California soil of the Petri dishes. It is also to be seen from the photographs that the characteristic efficiency at cellulose destruction of the California soil while somewhat abated by its removal to other climates, is none the less still clearly persisting there. A part of this observation is entirely in accord with the results of McBeth's painstaking studies on cellulose destroying organisms and their comparative effects in soils from different climates, a fuller account of which than heretofore given is soon to appear.<sup>7</sup>

Considering the plates above referred to a little more in detail, it is of interest to follow the changes in capacity to dissolve cellulose which appear to result from the removal of a soil from its own climate to another. In making such comparison it should be borne in mind that at the beginning of the experiment the surfaces of all the Petri dishes looked alike and indeed very much like No. 6 in either Plate D or Plate E, or perhaps of even more uniform whiteness. A glance at Plate A, which portrays the six soil samples from California of the California soil shows the marked power possessed by these soils to dissolve cellulose. This power

appears to be as marked in the "disturbed" soil at California as in the "undisturbed" soil or at least the qualitative method here employed allows of the detection of no difference between the two. It is striking to note moreover in Plate A that the second and third foot of the California soil at California dissolves cellulose as vigorously as does the first foot.

In striking contrast with the behavior of the California soil at California as regards cellulose dissolution as portrayed in Plate A is the behavior of the same soil at Maryland and at Kansas in respect to the same factor as illustrated in Plate B. Not only does the California soil at Maryland exhibit an extremely feeble power to dissolve cellulose in the first foot, but such power is virtually non-existent in the second and third foot. In other words, removal of the California soil to Maryland and its sojourn there for seven years have been instrumental in depriving it almost entirely of its cellulose destroying powers. At Kansas the California soil has not fared so badly in the surface foot and still shows itself fairly vigorous at the destruction of cellulose. But in the second and third foot and particularly in the latter, the solvent power of the soil for cellulose is extremely feeble and possibly wanting. The black blotches in Figs. 5 and 6 of Plate B, are contaminating moulds with no solvent effects on cellulose. Such black blotches are not to be confused with the dark soil color showing through the paper as in Fig. 4, Plate B.

A study of Plate C shows that the Kansas soil becomes more active at cellulose destruction in the first foot as a result of its removal to California. Owing to the black mould which contaminated Fig. 2 in Plate C, it is impossible to say if the effect just described has descended to the second foot or not. At its home location for some unaccountable reason the Kansas soil has but little cellulose destroying power in the second foot, and yet shows a fair power in that direction in the third foot. It does not possess such power in the third foot at California. At Maryland the Kansas soil, like the California soil, appears to have lost almost in its entirety its cellulose destroying power, though possibly a remnant thereof still remains in the surface foot. These observations are borne out by Figs. 4, 5 and 6 in Plate D.

Plate D, Figs. 1, 2 and 3 illustrate the behavior in respect to cellulose destruction of the Maryland soil at California in which cellulose destruction is fairly vigorous particularly in the first two feet and slight in the third foot. When one contrasts the foregoing with Figs. 1, 2 and 3 on Plate E, which exemplifies the results obtained with the Maryland soil at Maryland, it becomes clear how strikingly altered in the respect noted the Maryland soil at California has become. From a feeble power or none at all to destroy cellulose, it has developed at California a fairly vigorous power. At Kansas the Maryland soil has also attained some power at cellulose destruction in the first foot. In the second and third foot it has remained virtually unchanged even at Kansas. Even in the



first foot its cellulose destroying power is not as great as at a corresponding depth of the same soil at California.

#### THE CHEMICAL INVESTIGATIONS.

There will be found in the introduction to this paper an account of the general plan of the chemical investigations. The most important of these was of course the analysis by the official strong acid digestion method of every soil represented in these studies. The results obtained in these studies are therefore given first and include besides the analysis of the hydrochloric acid extracts for the constituents noted, the determinations of phosphoric acid by the nitric acid extraction method (gravimetrically) and the determination of potash by the perchloric acid method as modified by Davis.<sup>2</sup> The results are given in Table VIII.

In order to compare the different constituents shown by analysis of the different soils, as variously placed, to best advantage we shall consider every cognate group separately so far as possible.

##### *Insoluble and Soluble Silica.*

In general the data for insoluble and those for soluble silica as obtained by us for the different soils appear to be in harmony with Hilgard's observations on the effects of climate on the constituents of soils here under consideration. In some respects however, our data are at variance with Hilgard's on the same subject. Specifically, we find that the California soil remains virtually unchanged with respect to its content of silica (both insoluble and soluble) when it is disturbed for three feet in depth, but again replaced in the same position. When, however, it is placed at Kansas it gains very markedly in its content of insoluble silica and loses as markedly with respect to its soluble silica content. The Maryland climate seems to exert its effects again irregularly. For example the California soil while not gaining nearly as much there in insoluble silica as it does at Kansas still shows a very appreciable gain over the same soil at California, but it also gains very appreciably in soluble silica whereas the opposite effect would naturally be expected.

Unexpectedly, again the Kansas soil gains in insoluble silica when placed at California, though to be sure the gain is not great. In soluble silica, again the unexpected occurs and there is a marked decrease in that constituent when the Kansas soil is placed at California. When placed at Maryland, on the other hand, the Kansas soil remains practically unchanged with respect to insoluble silica, but loses very appreciably in its soluble silica content. All of these observations are of course made on the basis of the three-foot columns.

The Maryland soil is characteristically and expectedly high in insoluble silica and low in soluble silica. It is very slightly changed in either respect by being placed at either California or at Kansas. Some changes

worthy of mention do however occur. For example, the insoluble silica appears to remain unaltered in quantity in the Maryland soil or virtually so by its placement at California, but a distinct increase in soluble silica is clearly accompanied therewith. On the other hand, at Kansas the Maryland soil increases appreciably in insoluble silica and suffers a decrease in soluble silica. It will be remembered that the California soil is similarly affected at Kansas, except more strikingly.

TABLE VIII.  
CHEMICAL ANALYSES OF SOILS STUDIED BY OFFICIAL METHOD.

	California Check.			Cal. in California.			Cal. in Kansas.			Cal. in Maryland.		
	1st ft.	2nd ft.	3rd ft.	1st ft.	2nd ft.	3rd ft.	1st ft.	2nd ft.	3rd ft.	1st ft.	2nd ft.	3rd ft.
Insol. Sil.	60.31	61.21	61.82	61.98	61.04	60.70	70.01	68.70	75.20	63.23	64.20	65.30
Sol. Sil.	13.09	15.88	16.94	11.78	13.12	17.93	7.73	10.88	6.80	16.92	16.36	15.14
K <sub>2</sub> O	.51	.42	.51	.47	.49	.43	.41	.51	.47	.42	.47	.43
Na <sub>2</sub> O	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
CaO	.58	.66	.59	.59	.72	.61	.79	.74	.....	.74	.87	1.96
MgO	.....	2.83	.89	1.36	3.40	1.48	1.23	3.35	2.56	1.15	1.75	1.08
Mn <sub>2</sub> O <sub>4</sub>	.18	.18	.14	.18	.12	.18	.46	.51	.40	.52	.65	.28
Fe <sub>2</sub> O <sub>3</sub>	7.99	8.43	8.14	9.00	7.99	8.88	2.22	3.55	3.70	6.36	3.25	5.62
Al <sub>2</sub> O <sub>3</sub>	4.24	7.27	3.18	4.80	7.99	2.86	12.46	6.85	5.88	4.20	8.59	5.46
P <sub>2</sub> O <sub>5</sub>	.13	.14	.13	.12	.13	.14	.17	.17	.16	.15	.17	.17
SO <sub>3</sub>	.10	.12	.09	.10	.10	.08	.10	.06	.05	.14	.11	.09
Volatile matter	..	2.37	6.49	8.66	4.65	6.43	4.18	4.63	4.27	5.39	3.17	4.22

	Kansas in Cal.			Kan. in Kansas			Kan. in Maryland.		
Insol. Sil.	64.52	63.33	61.44	63.85	60.40	60.81	60.66	61.92	62.32
Sol. Sil.	13.64	15.94	17.80	18.28	18.74	17.74	15.16	14.54	13.73
K <sub>2</sub> O	.61	.58	.81	.68	.71	.83	.59	.62	.74
Na <sub>2</sub> O	.....	.....	.....	.....	.....	.....	.....	.....	.....
CoO	.61	1.30	2.06	.84	1.08	1.66	1.08	.66	.66
MgO	.95	.94	.78	.92	1.01	.91	3.84	2.33	4.66
Mn <sub>2</sub> O <sub>4</sub>	.14	.13	.13	.61	.43	.....	.28	.36	.23
Fe <sub>2</sub> O <sub>3</sub>	6.95	6.66	5.47	3.40	3.40	2.96	6.95	4.19	8.73
Al <sub>2</sub> O <sub>3</sub>	5.67	5.98	8.43	8.14	10.76	10.84	6.85	9.41	5.39
P <sub>2</sub> O <sub>5</sub>	.11	.13	.10	.15	.13	.15	.15	.14	.12
SO <sub>3</sub>	.11	.09	.07	.11	.10	.17	.11	.12	.12
Volatile Matter	6.08	4.39	2.35	2.26	2.41	3.64	3.60	4.83	2.84

	Maryland in Cal.			Md. in Kansas			Md. in Maryland.		
Insol. Sil.	79.10	78.31	83.01	84.08	80.24	81.88	81.88	73.87	79.96
Sol. Sil.	6.80	7.58	.....	4.13	5.73	5.89	5.89	11.18	3.39
K <sub>2</sub> O	.24	.16	.28	.31	.26	.19	.28	.31	.28
Na <sub>2</sub> O	.....	.....	.....	.....	.....	.....	.....	.....	.....
CoO	.28	.22	.20	.26	.15	.20	.20	.15	.13
MgO	.51	.38	.18	.28	.29	.21	.21	.23	.19
Mn <sub>2</sub> O <sub>4</sub>	.16	.19	.12	.12	.11	.16	.16	.06	.10
Fe <sub>2</sub> O <sub>3</sub>	6.06	6.95	7.54	2.66	2.96	2.81	2.81	2.51	2.79
Al <sub>2</sub> O <sub>3</sub>	2.73	3.65	4.62	6.22	7.76	7.35	7.35	7.95	9.97
P <sub>2</sub> O <sub>5</sub>	.10	.14	.17	.10	.15	.11	.15	.09	.13
SO <sub>3</sub>	.09	.08	.05	.12	.09	.13	.13	.09	.09
Volatile Matter	3.37	2.09	3.72	1.61	2.21	.66	.66	3.42	2.97

In general, it appears to be true despite the puzzling irregularities noted with respect to the insoluble and soluble silica that the silica as a whole seems to be in smaller quantity at California in any of the soils, and the soluble silica in greater quantity than at the other stations. In that respect therefore Hilgard's observations are confirmed. Just why, however, the smaller amount of leaching which should occur at Kansas than at Maryland should result in a higher insoluble silica content at Kansas is not clear to us and certainly is not capable of simple explanation.

#### POTASH.

The disturbance of the California soil at California causes a slight loss of potash, but its sojourn at Kansas or at Maryland is almost without effect in that respect, though possibly a slight loss occurs at Maryland when averages in all cases of the three-foot column are considered.

The Kansas soil loses very considerably in the average for the three-foot column by being placed either at California or at Maryland. The loss amounts to more than one-sixth of the total amount of acid soluble potash now in the Kansas soil at Kansas and is only slightly greater at Maryland than at California.

The Maryland soil like the Kansas soil loses potash in the average of the three-foot column by being placed either at California or at Kansas. The loss is, however, greater at California than at Kansas and amounts to more than one-fourth the acid soluble potash present now in the Maryland soil.

It may almost seem needless to remark that the losses in potash suffered by both the Kansas and the Maryland soils in California are difficult to account for, owing to the much smaller degree of leaching which occurs there than at other stations. In dealing with acid digestion of soils, however, it is possible that we introduce an error which may account for the differences between the potash content of the different soils which are above noted.

#### LIME AND MAGNESIA.

##### *The California Soil.*

The figures given above for lime and magnesia in the soils studied are as interesting as they are unexpected. For example, while the disturbance of the California soil as explained at its own location increases slightly the lime content, the increase is almost slight enough to be accidental; but when the same soil is maintained at Kansas as explained for seven years, it gains considerably in lime content in the first two feet. This is surprising since the superior conditions which we assume exist at Kansas for the promotion of leaching processes should give results opposite to those noted. When we consider the much more marked increase in lime which characterizes the same soil when placed at Maryland, we

are at a loss for a simple and ready explanation for the wholly unexpected fact.

The magnesia on the other hand is scarcely diminished in the California soil when the latter is allowed to remain at Kansas for seven years. At least this is so for the first two feet of soil in depth, while the third foot even receives an accretion of magnesia. When the same soil is placed at Maryland, however, the opposite effect of that noted in the case of the lime occurs, and the magnesia content particularly in the second and third foot of soil becomes much reduced in quantity.

#### *Kansas Soil.*

When the Kansas soil is placed at California it loses about 33 1/3 per cent of its lime in the first foot, but gains about as much in the second foot and nearly as much in the third foot. At Maryland we have again the unexpected and find a gain in the lime content of the Kansas soil of more than 25 per cent in the first foot, but a decided loss in both the second and third foot of the column.

With reference to magnesia the conditions are again different. The Kansas soil placed at California gains very slightly in the first foot, loses slightly in the second, and loses very decidedly in the third foot. When the same soil is placed at Maryland, however, an enormous increase in magnesia content in the soil occurs in seven years amounting to from three to five or more times the amount originally present in different parts of the soil column.

#### *Maryland Soil.*

Considering the relatively small amounts of both lime and magnesia which exist in the Maryland soil under its natural conditions it is interesting to note that it increases slightly in lime content when placed either at Kansas or at California. The increase is greatest at the latter place, and varies from .07 per cent to .08 per cent throughout the three-foot column. At Kansas the increase is found in the first and third foot, in which it amounts respectively to .06 per cent and .07 per cent, the original amount remaining stationary in the second foot.

With respect to its magnesia content, the Maryland soil shows much more evidence of change through its placement at Kansas and California (particularly the latter) than it does in the case of its lime content. Thus there is an increase in the magnesia content of the whole Maryland soil column at Kansas varying in magnitude from .02 per cent to .07 per cent. At California, however, while there is a possible slight loss of magnesia in the third foot of soil, there is a very large gain in the first and second foot of soil which amounts almost to a doubling of the original magnesia content.

## MANGANESE.

The manganese content of the California soil when placed at Kansas and at Maryland becomes very markedly increased. Thus at Kansas it becomes approximately three times that contained in the same soil at its natural location, and the increase is similar throughout the whole soil column in the same soil in Maryland. Whether this striking difference, which of course cannot be attributed to accident, is of significance, remains to be determined and further discussed later.

In the Kansas soil, on the contrary, we start with a high manganese content and evidently reduce it rapidly by placing the soil either at California or at Maryland, particularly at the former. The manganese content of the Kansas soil at California in the first foot, for example, is only one-fourth that at Kansas, and at Maryland it is less than one-half that at Kansas.

The Maryland soil at its natural location contains less manganese than the California soil at its home position. Nevertheless, when it is placed at Kansas it gains for the three-foot column .07 per cent  $Mn_2O_4$ , while at California it gains twice that amount in the same three-foot column. It appears now that even in this instance, in which the absolute quantities of manganese are small, the gains in that element experienced by the Maryland soil when placed for seven years at either California or at Kansas are above that of the experimental or accidental error, in magnitude.

## IRON AND ALUMINA.

Some very interesting phases of the iron-alumina content of soils as affected by climate are suggested by the unusual data obtained by us as above reported. It appears in general that placing an eastern soil at California insures a large gain in iron and almost a correspondingly large loss in alumina and vice versa. The magnitude of these gains and losses may be equivalent to one-half to one-third the quantity originally present in the soil which is transplanted from its natural location to another, as exemplified in the procedure above outlined.

Taking for a little closer examination one soil at a time we find that the mere disturbance at its own location of the California soil while it is instrumental in altering the content of iron and alumina somewhat in the soil column does not markedly change the magnitude of the quantity of each of the substances in question in that soil. When, however, we keep the California soil at Kansas for seven years we find that it loses from one-third to one-half its iron content and at the same time gains correspondingly or nearly so in alumina content. In Maryland the soil does not suffer so great a loss in iron as it does at Kansas, yet it does lose large amounts thereof. On the other hand, its gain in alumina, especially in the second and third foot, is about equally large.

The Kansas soil nearly doubles in the three-foot column in iron content by being placed at California, but it loses correspondingly in alumina content. Hence the combined iron and alumina content is not very different in that respect in the Kansas soil at the two stations. Just as the California soil loses in iron and gains in alumina when placed at Maryland, so does the Kansas soil gain and lose conversely. It must be remarked, further, however, and this may be significant, that the losses and gains involved are of about the same order of magnitude in Maryland, whether the soil affected be the Kansas or the California soil. Moreover, in the absolute, the losses or gains are smaller than those involved in moving the California soil to Kansas or vice versa.

The Maryland soil remains unaffected or virtually so in respect to its iron content by being placed at Kansas. It loses decidedly, however, in respect to its alumina content. To be sure, it should be added that the quantity of iron in the Maryland soil is very small. When, however, the Maryland soil is placed at California its iron content is more than doubled and correspondingly its alumina content is decreased by more than 40 per cent.

#### PHOSPHORIC ACID

Though the difference is not very great in the absolute, it is quite clear from the table that the relative differences between the phosphoric acid content in the California soil at California on the one hand, and at Kansas and Maryland on the other, are very considerable. More specifically it appears that the tendency is for the California soil to increase in phosphoric acid content when placed either at Kansas or at Maryland. The increase is about the same at both places and amounts to from .02 per cent to .05 per cent.

When the Kansas soil is placed at California it is similarly affected to the California soil placed at Kansas except that the change occurs in the opposite direction. Thus a slight loss is suffered by the Kansas soil when it is placed at California. On the other hand, it remains unchanged at Maryland. In other words, the effects produced by moving the California soil and the Kansas soil to other stations seem to confirm each other.

The Maryland soil data are again irregular in that the decrease in phosphoric acid which seems to be characteristic of soils placed at California does not occur when the Maryland soil is so placed. It is even possible that the Maryland soil gains slightly in phosphoric acid by being placed at California. It must be added, however, that the phosphoric acid figures are practically the same, within the limits of error, when the whole three-foot column of soil is considered in every case.

#### SULPHURIC ACID.

The sulphuric acid data are indeed very irregular. Thus the California soil gains in sulphuric acid content when placed at Maryland but

loses in that respect when placed at Kansas. When, however, the Kansas soil is placed at California it too loses in sulphuric acid, but as in the case of the California soil gains in that respect in Maryland. The Maryland soil behaves in respect to sulphuric acid like the Kansas soil in that it loses in that constituent when placed at California. At Kansas, on the other hand, the Maryland soil gains in sulphuric acid.

In general, it now appears that the sulphuric acid data are of no great significance. Moreover, the figures as discussed above are gathered into totals for the soil columns of three feet and on such small absolute amounts may lead to error in comparison. We do not therefore attach very much weight to the data submitted under the heading "Sulphuric Acid."

### HUMUS.

Since under humid conditions the humus of the first foot merely is of importance, the subsoil samples of these studies were not examined for humus and only the soil from the first foot of every column was studied. The Grandeau-Hilgard method for humus determination was employed. The following table indicates the percentages of humus found.

TABLE IX.  
PERCENTAGE OF HUMUS (GRANDEAU-HILGARD METHOD).

Depth.	California Soil.			Kansas Soil.			Maryland Soil.		
	In Cal.	In Kan.	In Md.	In Cal.	In Kan.	In Md.	In Cal.	In Kan.	In Md.
1st Foot.....	% 1.00	% 1.06	% 1.28	% 1.05	% 1.15	% .97	% 1.22	% 1.16	% 1.19

California soil "undisturbed" 1.02%

The variation in the percentage of humus in the different soils as shown in the foregoing table is not a great one and it is a matter of doubt if any significance may justifiably be attached thereto. Disregarding the latter point for a moment, however, it appears that the "disturbed" California soil had decreased in humus content over the check California soil, and that on the other hand the California soils both at Kansas and at Maryland had increased in humus content, the latter very appreciably, the former slightly. The Kansas soil, on the other hand, appears to have lost humus by its sojourn at both Maryland and California. While the loss at the latter station was to be expected on *a priori* grounds, the more marked loss at the former station is entirely unexpected. Another determination for humus on the same soil seemed only to confirm the earlier finding. As was true in other cases above discussed, the Maryland soil behaves irregularly with respect to the matter of its humus content. Thus it loses in humus content to the extent of .03 per cent by being placed at Kansas, but gains a similar amount at California.

## TOTAL NITROGEN IN THE SOIL.

The total nitrogen determinations on the soils studied were carried out by the method above cited and the results are set forth in Table X which follows.

TABLE X.  
PERCENTAGES OF TOTAL NITROGEN.

Depth.	California Soil.			Kansas Soil.			Maryland Soil.		
	In Cal.	In Kan.	In Md.	In Cal.	In Kan.	In Md.	In Cal.	In Kan.	In Md.
1st Foot. ....	% .096	% .092	% .089	% .128	% .141	% .120	% .121	% .110	% .099
2nd Foot. ....	.081	.065	.071	.088	.095	.086	.065	.064	.057
3rd Foot. ....	.067	.064	.057	.065	.057	.070	.064	.063	.056

"Undisturbed" soil: 1st ft. .096; 2nd ft. .084; 3rd ft. .072

It is quite clear from a study of the data submitted in the foregoing table, that mere disturbance of the California soil at its own location is powerless to alter materially its nitrogen content, even for a depth of three feet. For example, the "disturbed" and "undisturbed" soil contain exactly the same amount of nitrogen in the first foot. In the second foot the disturbed soil contains .003 per cent less than the other, but the latter contains almost an equal amount less in the third foot, so that the two columns remain virtually alike in their nitrogen content.

When the California soil is placed at Kansas or at Maryland, however, a very appreciable change takes place in the direction of lowering the nitrogen content of the soil. This change is rather slight in the first and third foot of soil but much more marked in the second foot. Therefore when the whole three-foot column is considered the California soil loses considerable quantities of nitrogen under the climatic conditions which obtain either at Kansas or at Maryland in the short space of seven years. The loss for the three-foot column is appreciably greater in the case of the soil at Maryland than in that at Kansas.

The Kansas soil loses more nitrogen by being placed elsewhere than does the California soil when it is so treated. This is true for both the first and the second foot, but not for the third foot. The loss noted is greater in the case of the Kansas soil at Maryland than in that at California, but the difference, though distinct, is not great. In the third foot the Kansas soil gains both at California and at Maryland in nitrogen, the greater gain being at Maryland.

Unlike the other two soils, the Maryland soil gains in nitrogen by being placed either at Kansas or at California. This is true for the whole three-foot column. The gain is very considerably higher at California than at Kansas, but that is only true for the first foot, the other two being about alike in both cases. It is deserving of notice in the case of the Kansas and the Maryland soils that the percentage of nitrogen decreases



much more abruptly from the first to the second foot than it does in the case of the California soil.

In general, the data for the total nitrogen in the soils studied are not indicative of changes in any one direction wrought by climate as a determinant. For a given soil it appears that removal of an arid soil to more humid regions means a decided decrease in nitrogen. For another soil from another region the opposite may be true, or at different stations at two extremes of climate the effect may be in the same direction on a soil introduced into both. In other words, we appear again to be dealing in our nitrogen values (as submitted in the foregoing table) with algebraic sums of quantities of nitrogen, on the one hand added by nitrogen fixation of various kinds, and on the other hand of nitrogen losses of various kinds. It is of course clear that these two kinds of effects need not operate uniformly in all cases. Thus high nitrogen fixation may be offset by rapid nitrification followed by leaching, or denitrification, and the net gain be a very small one. Conversely, rapid loss of nitrogen by oxidation of organic nitrogen or destruction through vigorous action of cellulose destroying flora may be offset wholly or in part by nitrogen fixation. In view of the foregoing facts and observations, it appears impossible to make any general statement, whose validity may be unquestioned, as to the effect of climate on the nitrogen content of soils. It seems possible, however, to predict from known characteristics of a given soil and a knowledge of the conditions under which it is to be placed, whether it will decrease or increase in its nitrogen content.

#### REACTION OF THE SOILS.

The California and Kansas soils at all stations give a neutral or alkaline reaction to litmus paper. This is of course to be expected on the basis of the high calcium content of these soils. The Maryland soil, however, acts very differently and shows even by the litmus paper test some very marked changes due to climatic effects. For example, at its home station the Maryland soil shows a distinctly acid reaction in the surface foot and a decidedly acid reaction in the second and third feet. When placed at Kansas, however, the reaction of the first foot changes to a neutral one and that of the second and third feet still remains acid. At California, however, a further change occurs and the reaction of both the first and the second foot changes to neutral or slightly alkaline to litmus paper and remains acid only in the third foot.

#### WATER EXTRACT STUDIES.

In order to gain some insight into the situation with respect to the water soluble constituents of the soils here studied we prepared water extracts by the following method: Two hundred grams of soil were digested with 400 c.c. of distilled water for six days, during which time they were shaken up several times a day. At the end of the period named

the extracts were filtered, 50 c.c. portions representing 25 gm. of soil were evaporated in platinum dishes to dryness and brought to a constant weight at 100° C. The total residue was then weighed and the dishes ignited at a low red heat to dissipate the volatile matter. This made possible the determination of both the volatile and non-volatile fractions of the water extract. The non-volatile residue was then taken up in water, and detailed analyses will be made of these which are to be reported later. It suffices here merely to discuss the data given in Table XI which give the amounts in per cent of the volatile and non-volatile matter in the water extracts.

TABLE XI.  
PER CENT WATER-SOLUBLE MATTER IN SOILS.  
From Exchange Plot Experiment.  
*Series A—Total Volatile Matter.*

Depth.	California Soil.			Kansas Soil.			Maryland Soil.		
	In Cal.	In Kan.	In Md.	In Cal.	In Kan.	In Md.	In Cal.	In Kan.	In Md.
1st Foot. ....	.0212	.0180	.0336	.0160	.0176	.0192	.0264	.0232	.0132
2nd Foot. ....	.0168	.0236	.0388	.0180	.0348	.0184	.0196	.0332	.0128
3rd Foot. ....	.0248	.0404	.0580	.0180	.0224	.0236	.0152	.0208	.0172

*Series B—Total Inorganic Matter.*

1st Foot. ....	.0204	.0264	.0284	.0392	.0336	.0216	.0144	.0256	.0128
2nd Foot. ....	.0120	.0232	.0292	.0204	.0372	.0148	.0268	.0240	.0108
3rd Foot. ....	.0152	.0344	.0376	.0264	.0284	.0228	.0208	.0100	.0128

The results given in the foregoing table are very interesting and in our opinion of great significance in connection with the general question of concentration of the soil solution. Considering one soil at a time, it appears clear that the California soil increases enormously in water soluble matter both volatile and non-volatile by being placed under more humid conditions. Such increase may frequently be equivalent to nearly 100 per cent of the amount originally present. The gain in the respect noted is greater in all cases for the California soil at Maryland than at Kansas.

The Kansas soil when the soil column is considered, loses in water soluble volatile and non-volatile matter at California, and at Maryland.

The loss at California is in keeping with the climatic effects as brought out in the case of the California soil, but the loss at Maryland is diametrically the opposite of the expected result and remains to be explained when the more detailed analyses have been carried out.

The Maryland soil gains decidedly in water soluble matter at both Kansas and California. This fact seems to render nugatory any attempt to explain the existence of certain quantities of water soluble matter in soils on the basis of simple climatic effects, since the Maryland soil and its behavior negatives a possible conclusion from the California soil and its behavior. Thus the California soil gains in water soluble matter when moved to more humid climates, while the Maryland soil gains in the same

regard when placed under more arid climates.

While, therefore, no simple relationships can be discovered, and certainly no direct ones between climate and the amount of water soluble matter in soils, in the data we have above submitted and discussed, they are beyond cavil of great significance in other respects. In the first place, they lend further confirmation to the ideas expressed by other soil chemists that the concentration of the soil solution is not uniform for all soils, as has been claimed by some. In the second place, the figures clearly indicate that climate exerts a profound effect on the concentration of the soil solution even though such effect may be in opposite directions on two different soils. In the third place, our results show that volatile constituents of the soil water increase and decrease with the non-volatile portions, or vice versa. Lastly, it may be of significance that the quantities of the volatile and of the non-volatile matter, in the absolute, are very considerable and of about the same order of magnitude, in general.

COMPARISON OF OUR CHEMICAL DATA WITH THE PARTIAL DATA  
OBTAINED BY SHAW AND WALTERS.

The following table is quoted from the bulletin by Shaw and Walters above referred to for the purpose of making a partial comparison of the chemical constitution of the soils here studied as they were five years ago and as they are to-day. It is obvious, as above indicated, that such comparisons must be taken *cum grano salis* since the methods employed in the analytical work were somewhat different, and the personal equation involved in the change in analysts cannot be overlooked.

TABLE XII.  
PERCENTAGE COMPOSITION OF SOILS.

*California Soil.*

	Nitrogen.	Phosphoric acid.	Potash	Sulphuric acid.	Lime.	Magnesia.
1st Foot.....	.118	.161	.49	.121	.704	.703
2nd Foot.....	.074	.127	.473	.053	.621	1.69
3rd Foot.....	.058	.124	.462	.046	.683	1.48
Average .....	.083	.137	.475	.073	.669	1.29

*Maryland Soil.*

	Nitrogen.	Phosphoric acid.	Potash	Sulphuric acid.	Lime.	Magnesia.
1st Foot.....	.111	.114	.342	.13	.191	.42
2nd Foot.....	.076	.127	.278	.111	.222	4.75
3rd Foot.....	.042	.111	.450	.053	.204	4.72
Average .....	.076	.117	.357	.098	.205	3.927

*Kansas Soil.*

	Nitrogen.	Phosphoric acid.	Potash	Sulphuric acid.	Lime.	Magnesia.
1st Foot.....	.174	.160	.602	.14	.89	1.301
2nd Foot.....	.121	.173	.783	.08	.796	2.29
3rd Foot.....	.040	.165	.902	.09	1.69	2.18
Average .....	.111	.166	.762	.10	1.125	1.92

NOTE.—The "Official Methods" recorded in Bulletin No. 107 (revised) of the Bureau of Chemistry, U. S. Dept. of Agriculture, were followed in making the analyses recorded in this paper.

## NITROGEN.

Beginning with the nitrogen consideration, it appears that the California soil has changed considerably even at its natural location with regard to its content of nitrogen. The change seems, however, to have been more in the nature of a redistribution of the nitrogen in the different soil layers than one of loss. This idea appears to receive support from the fact that the average nitrogen content of the three-foot column of the California soil is .083 per cent by the earlier analysis and is .081 per cent by our analysis. The redistribution of nitrogen referred to seems to have consisted in an increase of nitrogen in the second and third foot and a decrease in the first foot. At other points than at its natural location the California soil has in addition to the redistribution of its nitrogen suffered a loss of nitrogen as above explained.

The Kansas soil has suffered a loss of nitrogen equivalent to more than six times the loss suffered by the California soil, even if the average for the three-foot column is considered, and in addition has been subjected to a greater redistribution of nitrogen from the first foot into the subsoil. The average for the three-foot column by the earlier analysis is .111 per cent, while the average for the same column at its home location as found by us is .098 per cent. The loss suffered by the Maryland soil between the time of the Shaw and Walters' analysis and that of our analysis is midway between that of the California soil and that of the Kansas soil, if again the three-foot column of soil at its home station is considered. The averages for the two analyses in the order mentioned are .076 per cent and .071 per cent. The redistribution of nitrogen from the upper to the lower layers has also taken place in the Maryland soil as in the others.

In only two cases in the whole series of analyses do we note an increase in the average nitrogen content of the three-foot column, and they are both in the Maryland soil. One is in the case of the Maryland soil at California in which the average net gain for the three-foot column is .006 per cent and the other is in the case of the Maryland soil at Kansas in which the corresponding gain is .003 per cent.

## PHOSPHORIC ACID.

With regard to phosphoric acid the California soil has altered but slightly at its own station for the three-foot column, but again as in the case of the nitrogen it has lost slightly in phosphoric acid content, and a redistribution has occurred which has rendered the surface foot considerably poorer than it was and the other two feet each somewhat richer in phosphoric acid than they were five years ago. By being moved to Kansas and to Maryland, however, and left there for the period stated, the California soil has increased in phosphoric acid content very considerably as above explained.

The Maryland soil at its home location has lost for the three-foot column about .014 per cent  $P_2O_5$ , which is a very considerable amount. The loss has all been from the subsoil, since the surface foot has even gained .02 per cent  $P_2O_5$ , thus showing the opposite tendency from the California soil. The Maryland soil at Kansas, however, has not lost any phosphoric acid during the five years and has even gained slightly, as has the California soil at both Kansas and Maryland. At California, however, the Maryland soil has lost very large quantities of phosphoric acid even when the whole three-foot column of soil is considered, the loss amounting as an average for the column to .044 per cent  $P_2O_5$ .

The Kansas soil at its home location has lost .023 per cent  $P_2O_5$  as an average of the three-foot column in five years, the loss having been greatest in the second foot, but appreciable in both the first and third feet as well. At California, however, the Kansas soil has lost even more phosphoric acid and it amounts to .053 per cent as an average for the three-foot column there. At Maryland the Kansas soil has also lost more heavily in phosphoric acid than at home, but not nearly as much as at California, the average loss for the column being .03 per cent.

#### POTASH.

The California soil has lost very slightly in its potash content in a period of five years when averages for the three-foot column are compared. There has, however, been a greater redistribution within the column itself in potash content. The changes occurring, if any, in respect to the potash content of the California soil at other stations are noted under the heading "Potash" above.

The Maryland soil has lost in the period noted .069 per cent  $K_2O$  as an average for the three-foot column at its home station and very much more at the other stations as explained above.

The Kansas soil, like the California soil, loses but slightly in potash content in five years when an average of the three-foot column at its home station is taken for comparison.

#### SULPHURIC ACID.

The data for the two analyses of the California soil at California indicate a decided gain in sulphuric acid during the past five years, and even a greater gain in that respect for the same soil placed at Maryland. At Kansas, however, the sulphuric acid content of the soil appears to remain about the same with perhaps a very slight loss.

The Maryland soil shows a very slight gain in sulphuric acid at its home location, but a more marked gain in that respect at Kansas. At California, however, a loss occurs which is more decided than the gain at Kansas.

The Kansas soil gains very decidedly at Kansas in sulphuric acid con-

tent for the average of the three-foot column. The gain occurs in both the second and third foot, while the first foot shows a loss. The gain in sulphuric acid at Maryland is appreciable, but not so great as at Kansas. Again the gain occurs in the subsoil and a loss is noted in the surface foot. At California a slight loss occurs in the three-foot column of the Kansas soil, the loss being in the first and third foot, a slight gain being noted in the second foot.

On the whole, it appears that accretions of sulphur have been received by most of the soil from some source outside of the three-foot column of soil studied, if the gain where occurring may not be explained on the basis of a change in the quantities of other constituents in a given weight of the soil.

#### LIME.

Most striking of all the changes noted in the soils in question as revealed by the two analyses carried out in 1910 and in 1915 respectively, are those pertaining to their lime and magnesia content. Considering the average lime content of the three-foot column, it appears that the California soil at California has lost about .05 per cent of lime, including total calcium of the soil. While such loss is appreciable, it can not be said to be great. The redistribution of the calcium in different layers of the soil however, has been more marked. Thus the surface foot of soil has lost .11 per cent calcium and the third foot has lost more than .07 per cent calcium while the second foot has gained nearly .10 per cent calcium.

In the case of the Kansas soil at Kansas we find a slight gain of  $\text{CaO}$  for the average of the three-foot column in five years amounting to about .06 per cent. Within the column we find a loss of .05 per cent in the first foot, a gain of .28 per cent in the second foot, and a gain of .03 per cent in the third foot.

The Maryland soil is not subject to such striking and inexplicable changes as the other soils, possibly because of its initially very low lime content. Nevertheless, at its home location it loses in the five year period mentioned nearly .05 per cent  $\text{CaO}$  for the average of the three-foot soil column. The loss occurs in the second and third foot of soil, the surface foot remaining almost stationary in  $\text{CaO}$  content, and perhaps even gaining slightly.

#### MAGNESIA.

The magnesia content of the soils here studied is subject to greater change, speaking generally, than the lime content in them, when analyses of 1910 and 1915 are compared. Thus the average percentage of magnesia in the California soil for the three-foot column in 1910 was 1.29, whereas now it has increased to 2.03 per cent. The increase occurs in the first and second foot only, the third remaining the same as before.

Most of the gain is in the second foot, however. The Kansas soil at its home location loses very heavily in magnesia (considerably more than half) as an average for the three-foot column in a period of five years. The loss is almost uniform throughout the three-foot depth of the soil. Considering now the Maryland soil it appears almost unbelievable to note the enormous losses in magnesia which that soil has suffered at its home location in five years. Starting with an average magnesia content of 2.30 per cent for the three-foot soil column, it now contains, after five years only .21 per cent, indicating a loss (occurring in all depths) amounting to over 90 per cent of the total quantity present.

#### GENERAL THEORETICAL AND OTHER CONSIDERATIONS.

Lack of space in this paper forbids an extended theoretical discussion of the causes underlying the effects above shown and discussed which result from climatic influences on soils. Some of the effects noted are easily explicable on the basis of present knowledge; others are but difficultly so, if at all capable of being explained in simple terms. Some features of the results obtained stand out quite clearly, however, despite their unexpected nature in some instances. For example, it appears that the total internal surface of soils per given unit of dry weight increases with a decrease in precipitation or with an increase in aridity. This must of course exercise, as in general it appears to do, an important influence on the hygroscopicity, moisture equivalent, wilting point, tenacity, absorptive power, and many other physical characteristics of a given soil. The reason for such increase in internal soil surface under an arid climate in a soil fairly supplied with organic matter can now be sought only in theory. It is reasonable for example to argue that under the stresses and strains incident to extremes of moisture conditions in the soil of an arid region there would be a greater tendency toward the formation of aggregates or compound particles than in the same soil placed under humid conditions. The lack of leaching in the arid region would permit of the accumulation of cementing materials which would also operate to the same end. All of this would only occur in the arid region in the presence of a fair supply of organic matter, since without the latter the particles of silt and sand would tend to become compacted and cemented into masses of relatively small internal surface. If this theoretical consideration is allowed, and it may be that we shall be able later to throw light on its validity or invalidity through specific gravity and other studies, then the general trend of the physical data above submitted can be accounted for. The exceptional manifestations, however, can not be explained in simple terms and we are obliged to defer a more detailed consideration of them to such time and place as are more propitious for that purpose than the present.

With regard to the chemical data obtained by us, the theoretical considerations on the causes underlying them are more difficult than in the case of the physical data. This is so because of the great irregularity of some of the results obtained and further because of the fact that data given in percentages for any one constituent on the basis of a complete analysis, depend for their nature so much on the relative proportions of the other constituents present. The result is that it is very difficult to determine if losses or gains shown by percentages are real or merely apparent losses or gains. Here again, as in the case of the physical data we shall undoubtedly be better able to connect the results obtained with the causes underlying them when we shall have obtained more data on both the real and apparent specific gravities of the soils here studied. While leaching and its effects are clearly factors in the rearrangement of percentages of the different constituents of the soil, it does not follow that the climate which permits of the greatest amount of leaching is one posite may indeed be true, and further light for such facts must be sought in the laws of physical chemistry governing complex systems of solutions. With these in mind, the reversal of conditions with respect to losses of lime and magnesia in the same soil under the same conditions or even of iron or alumina or other constituents, may become much more intelligible. With regard to the nitrogen fraction of the soil, sufficient has probably been said above for the elucidation of the results obtained. To discuss more specifically the theoretical phases of the chemical data obtained would require much more space than is allowed us here, and we are therefore obliged to defer such discussion for other papers. It suffices to remark here that the data submitted by us clearly indicate profound changes in the chemical constitution of a given soil when it is changed from an arid to a humid climate or vice versa, and that even under a given set of climatic conditions a period of five years may produce very profound chemical changes in a given soil. Obviously, such chemical changes are not independent of either the physical and biological changes which accompany them.

On the biological side, the theoretical considerations are not unlike those of the physical and chemical sides of the question of climatic effects on soils. In general bacterial activities are far more pronounced under humid than under arid conditions, and so far as the California and Kansas soils are concerned, there is but one exception to this tendency, and namely cellulose destruction, which proceeds with much greater activity in the arid than in the humid region. Bacterial numbers on the other hand offer another exception in the case of the Maryland soil which increases in numbers of bacteria when moved from humid to arid conditions. The general trend, however, which is noted is probably to be accounted for in the decrease in the carbon supply of soils under arid



conditions and by the decrease in the water soluble inorganic and organic matter as attested to by the data above submitted. These, in general would affect all the bacterial activities but would operate particularly to deprive the nitrogen fixing and nitrifying bacteria of their source of energy. The latter class of bacteria, moreover, would be affected in other ways by the difference in absorptive surface (as affected by organic matter) for the ammonia produced by the ammonifying flora and thus would be poisoned by an excess of free unabsorbed ammonia directly, or inactivated by the large amount of soluble organic matter produced in the soil by the ammonia. It is clear that this effect would be most marked in the case of the nitrification of blood nitrogen, since the blood is so readily dissolved by the ammonia, and hence poor results with nitrification would be obtained. The opposite would of course be true under arid conditions. This theory has been advanced elsewhere<sup>5</sup> by the senior author to account for the non-nitrification of dried blood nitrogen in many arid soils when the blood is used in large quantities (1 per cent of the soil). In general, therefore, the theoretical conditions on the biological side of our problem seem to arrange themselves in a reasonable fashion tending toward the elucidation of the data obtained in the experiments.

Considering in conclusion the subject in the large, there can be no question that climate exerts in a very short space of time some very profound effects on a soil. It therefore appears questionable if after all, a tri-soil exchange plot experiment such as that which made our studies possible, gives for any length of time a true picture of what the same soil will do under three different climatic conditions. As we have seen, very deep seated changes in the soil occur with great rapidity when it is placed under a new set of climatic conditions. This would mean that wheat, for example, would not be grown on the same soil in three different localities, but on three different soils derived from one original soil under different climatic effects, and moreover that the differences would become more and more accentuated every year. It therefore seems, unjustifiable to assume that any differences in wheat grown under different climatic conditions on a soil supposedly the same in every climate, results merely from the climatic factor involved.

### SUMMARY.

A considerable part of detailed studies on changes in the physical, chemical, and biological nature of soils occurring through climatic effects is discussed. The studies were carried out on soils from the soil exchange plots used for the study of the composition of wheat in a cooperative experiment established in 1908 by the Office of Cereal Investigations, United States Department of Agriculture, the Maryland Agricultural Ex-

periment Station, the Kansas Agricultural Experiment Station, and the California Agricultural Experiment Station. Among many striking facts revealed through these investigations only a few can be mentioned here. The reader must study the main discussions and tables to obtain a clear view of the results.

1. Soils change markedly in color in a period of seven years, and perhaps less, when moved to other climates. For example, Kansas and Maryland soils at California become more deeply reddish in color; California and Kansas soils become bleached to a light gray or yellowish gray at Maryland. The differences are so great that samples of any one original soil from the three different stations to-day show no outward resemblance among themselves, but appear to represent three very distinct soil types.

2. In general the hygroscopic coefficient, the moisture equivalent, and the wilting point of any of the soils increase when the soil is placed at California. Some exceptions to this rule are noted.

3. In general bacterial numbers increase in arid soils placed under humid conditions. In general, also the opposite is true for humid soils. The Maryland soil offers an exception to the latter rule.

4. Ammonification, nitrification and nitrogen fixation follow the general trend of bacterial counts as described in the last section. The general trend, however, applies in the case of nitrification to certain forms of nitrogen only. In the case of other forms of nitrogen very peculiar conditions exist which are fully explained above.

5. Cellulose destruction by soils proceeds with greater rapidity under arid than under humid conditions with any given soil type. Cellulose destruction therefore appears to follow in general the opposite course of other micro-organic activity in soils as affected by climate.

6. Marked changes in the acid soluble constituents of soils are wrought by climatic effects. It is difficult to generalize with respect to them, but it may be said that soils may often obtain accretions of the different constituents when removed from one climatic environment to another. Thus, for example, the California soil increases in lime at Kansas, and Maryland, particularly at the latter station, and loses in iron. The general tendency is for soils to increase in iron and decrease in alumina when placed under arid conditions, and vice versa.

7. Phenomenal losses in certain constituents in five years seem to have occurred in some soils even when the latter were not moved. Thus for example the Maryland soil loses in the period named enormous quantities of magnesia.

8. Very interesting data are submitted on the total water soluble constituents in the soils studied. Large increases occur in the California soil in that respect when it is moved to the Kansas or Maryland stations.

On the other hand, the Maryland soil gains in water soluble matter when moved to Kansas or to California.

The brief summary given above is merely fragmentary and, as explained above, the reader must seek a true picture of the variety and extent of the data obtained in the main body of the text.

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**PLATE I**

**Figs. 1, 2 and 3, show cellulose destruction by 1st, 2nd and 3rd foot of California soil (undisturbed) at California.**

**Figs. 4, 5 and 6—Same for same soil disturbed.**



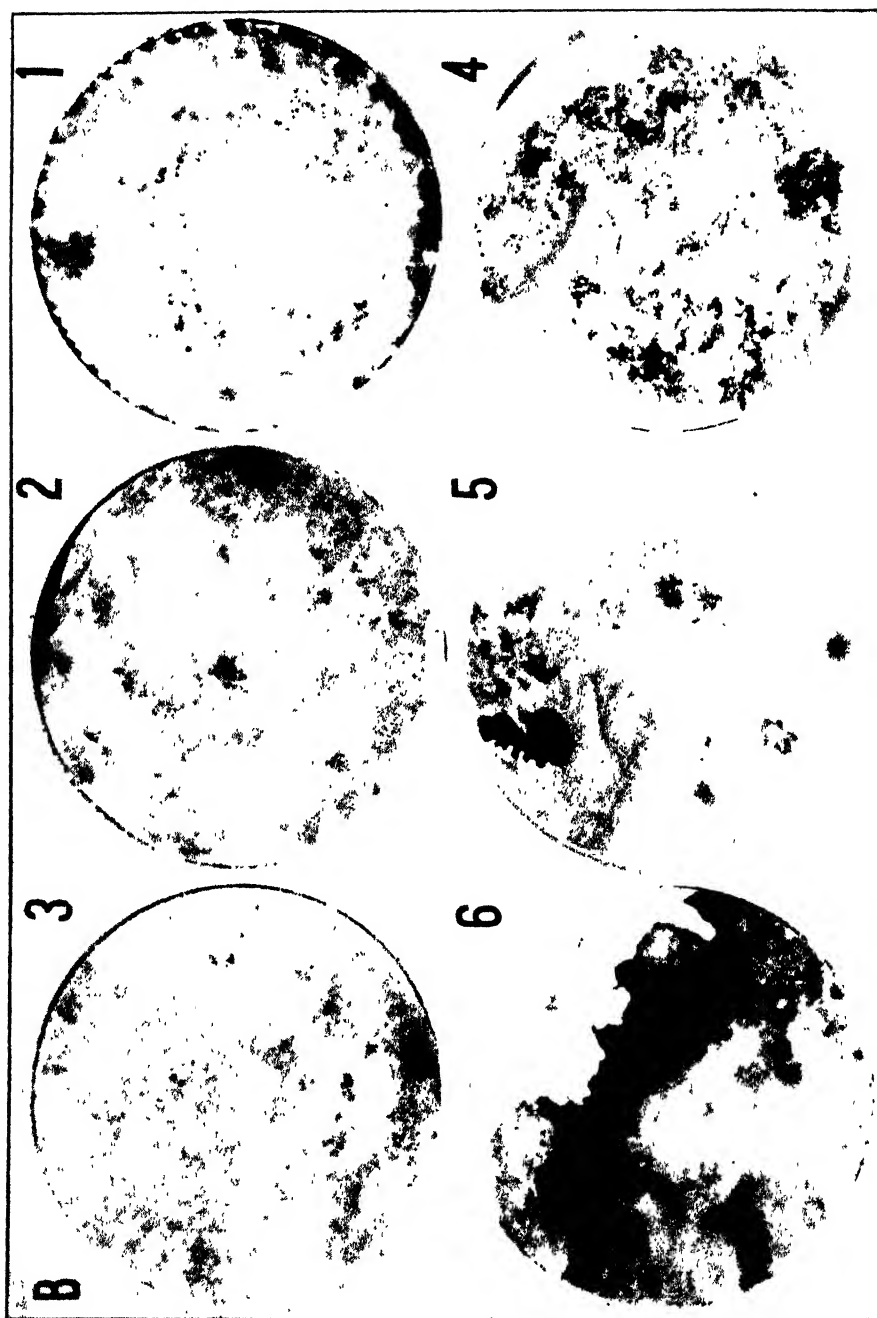


PLATE II

**Figs. 1, 2 and 3, show cellulose destruction by 1st, 2nd and 3rd foot of California soil at Maryland.**

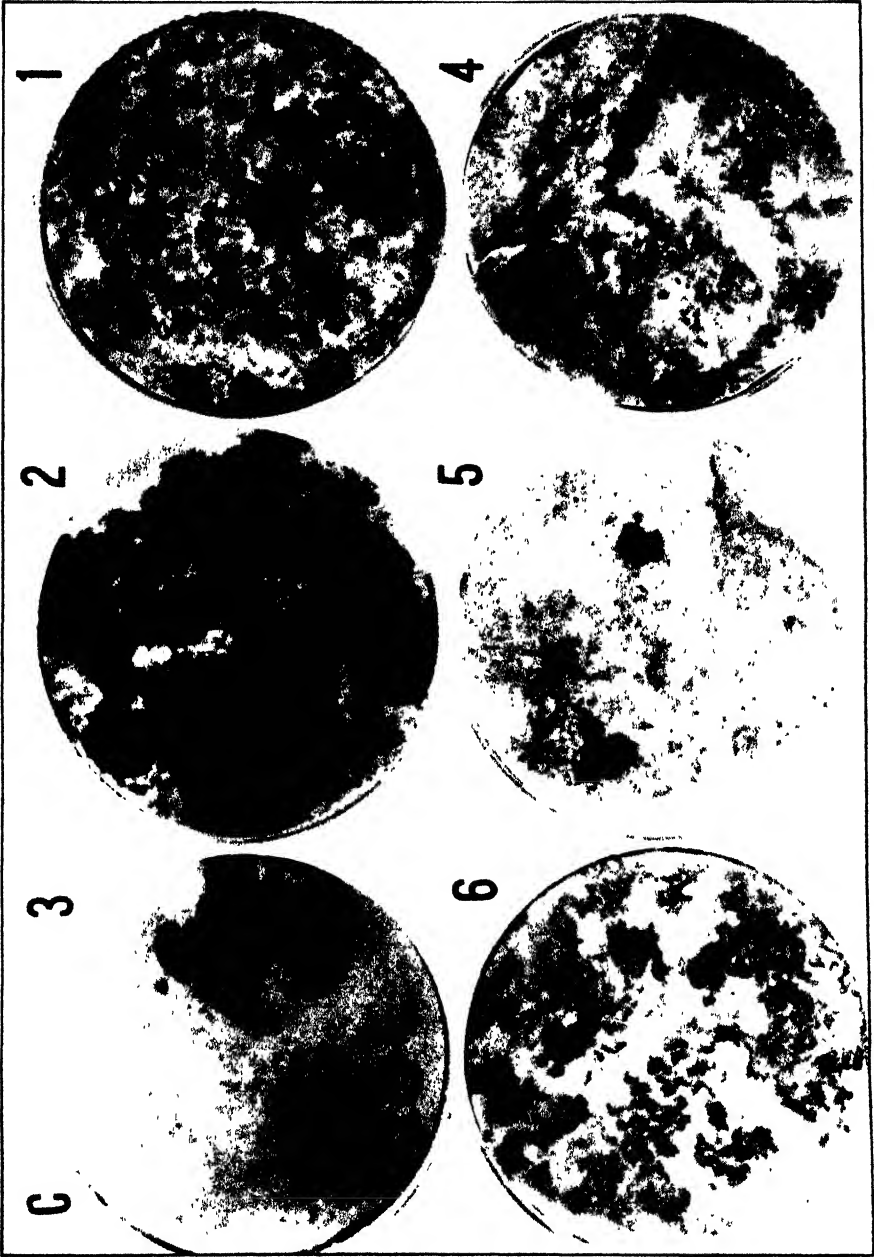
**Figs. 4, 5 and 6—Same for California soil as Kansas.**

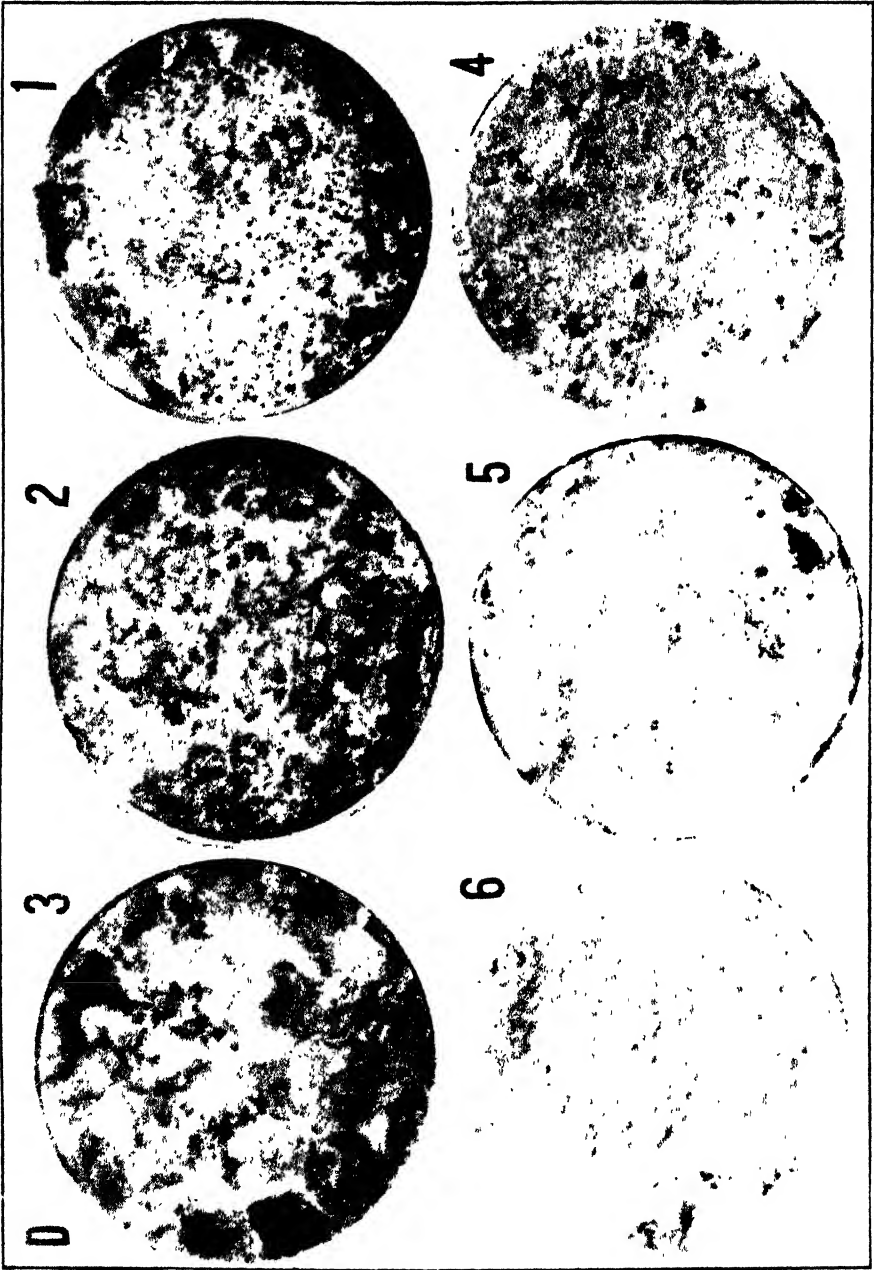


**PLATE III**

**Figs. 1, 2 and 3, show cellulose destruction by 1st, 2nd and 3rd foot of Kansas soil  
at California.**

**Figs. 4, 5 and 6—Same for Kansas soil at Kansas.**





#### PLATE IV

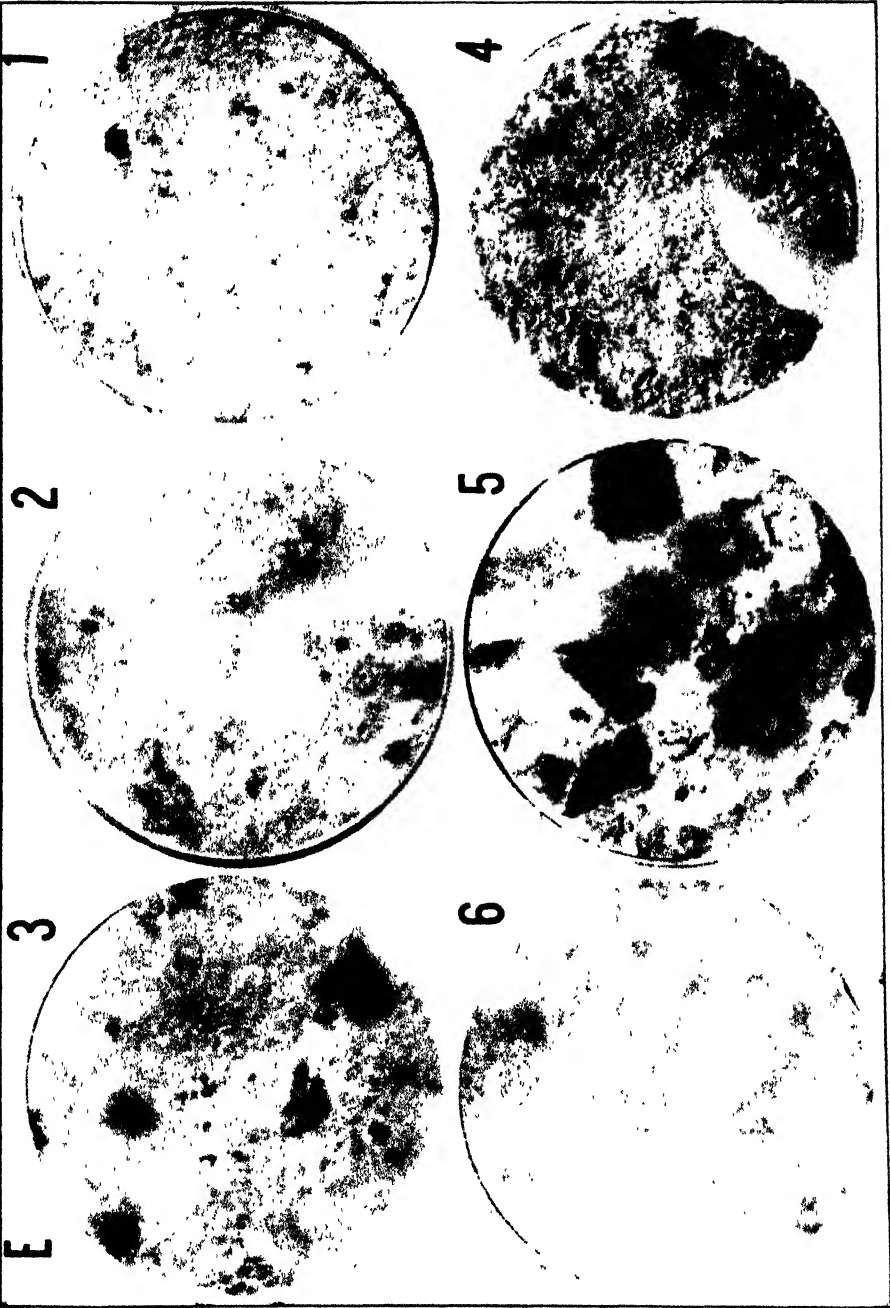
Figs. 1, 2 and 3, show cellulose destruction by 1st, 2nd and 3rd foot of Maryland soil at California.

Figs. 4, 5 and 6—Same for Kansas soil at Maryland.

## PLATE V

**Figs. 1, 2 and 3, show cellulose destruction by 1st, 2nd and 3rd foot of Maryland soil at Maryland.**

**Figs. 4, 5 and 6—Same for Maryland soil at Kansas.**





# THE INFLUENCE OF SOME COMMON HUMUS-FORMING MATERIALS OF NARROW AND OF WIDE NITROGEN-CARBON RATIO ON BACTERIAL ACTIVITIES.\*

By P. E. BROWN AND F. E. ALLISON.

The determination of the nitrogen-carbon ratio in soils is now coming to be considered of much importance in fertility studies. Not only does it show the organic matter content of soils more accurately than the more or less arbitrary humus determinations concerning which considerable difference of opinion exists, but it also throws some light upon the rate at which decomposition processes are occurring in the soil.

When applications of organic matter are made to the soil, the nitrogen-carbon ratio of the soil is modified to a greater or less extent, depending on the ratio of these elements in the materials applied. Stewart<sup>4</sup> has shown that the common humus-forming substances have a much wider ratio than soils, and hence the effect of turning under corn stover, oats straw or manure in a soil would be to widen the nitrogen-carbon ratio. The same author has also shown that under normal conditions the nitrogen-carbon ratio of the soil has a tendency to become narrower as the age of the organic matter increases. Other investigators have noted the same narrowing of the nitrogen-carbon ratio in decomposing organic matter and have concluded that it is due to the greater ease with which the carbonaceous portion of the organic matter is decomposed and disappears than is the case with the nitrogenous part.

Furthermore, as the more actively decomposable portions of the organic matter are removed, the remainder consists of rather inert materials whose decomposition proceeds, as would be expected, more slowly and with much more difficulty.

The presence, therefore, of a narrow nitrogen-carbon ratio in soils might be considered to show a deficiency in fresh organic matter and consequently a lack of the proper decomposition processes for the production of optimum amounts of available plant food.

This is actually the case in humid soils. Experience has shown that if the ratio narrows beyond a point of about 1 to 10, crop yields may be reduced, evidently because of an insufficient production of available nitrogen, phosphorus and potassium. On the other hand, if the ratio is 1 to 12 or above, bacterial activities apparently occur to a satisfactory extent and sufficient amounts of soluble plant food are produced for good crop growth.

The question now arises whether, when a soil shows a narrow nitrogen-carbon ratio and hence a lack of fresh organic matter, materials of

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\* From the Soil Chemistry and Bacteriology Laboratory, Iowa State College.



the widest possible ratio should be chosen to supply the deficiency. In other words, would the bacterial activities and crop yields be benefitted to as great an extent by additions of straw as by the turning under of a crop like clover which has a much narrower nitrogen-carbon ratio, but at the same time supplies more nitrogen to the soil?

It is commonly believed that clovers or other leguminous green manure crops are of much more value for supplying deficiencies of organic matter in soils than straws or stover, but the latter materials may be applied at a much less expense and if they will serve as well, that is if they will have the same or a better effect on bacterial activities and crop yields, they should be used.

Obviously, the nitrogen content of the soil should be considered in making a choice of materials to increase the organic matter content. When nitrogen is lacking, should leguminous crops be employed because of the nitrogen which they supply? Would it not be quite as satisfactory to increase the organic matter content of the soil and the decomposition processes by the use of a cheaper material which would increase the fixation of nitrogen from the atmosphere? Would these cheaper materials exert a sufficiently greater effect on bacterial activities especially on azo-fication or non-symbiotic nitrogen-fixation to prove as valuable as the leguminous green manures?

In other words, with the straws, would a sufficiently large increase in nitrogen content in the soil occur through azo-fication to keep the crop as well supplied with nitrogen as when the legumes were used? Again, would the nitrogen present in the soil be made available as fast by the decomposition produced by the straws as that present in the legumes is transformed by the decomposition which they engender?

These are the questions which arose from a consideration of the question of the nitrogen-carbon ratio in soils and which the experiments reported in the following pages were planned to answer.

Briefly then, it may be said that the purpose of this work was to study the influence of materials of narrow and of wide nitrogen-carbon ratio, when applied to soils low in organic matter, on certain bacterial activities. The processes studied were those which are important from the standpoint of the decomposition of nitrogenous organic matter, namely ammonification and nitrification, and that which concerns the increase in soil nitrogen, namely azo-fication or non-symbiotic nitrogen-fixation.

The comparative effects of these materials on the growth of oats in greenhouse pots were also studied in the attempt to ascertain whether the crop yields were affected in a manner similar to the bacterial processes and also to determine if possible, whether inexpensive materials of a narrow nitrogen-carbon ratio would not, upon undergoing decay, stimulate bacterial activities and especially increase sufficiently the fixation by the soil of nitrogen from the atmosphere, to give as satisfac-

tory yields as materials containing more nitrogen in proportion to the carbon present.

#### THE PLAN OF THE EXPERIMENT.

The soil chosen for this work was secured from one of the college experimental orchards and is classed by the Bureau of Soils as Miami sandy loam. It was low in organic matter and slightly acid in reaction showing a lime requirement, according to the Veitch method, of 736 pounds of calcium carbonate per acre of two million pounds of surface soil. Before the special treatments were made, therefore, sufficient calcium carbonate was applied to neutralize the acidity and bring the lime content of the soil up to two tons per acre.

After being sieved, air-dried and treated with lime as mentioned, the soil was filled into thirty-six earthenware pots in the greenhouse, at the rate of 36 pounds to the pot.

The special treatments of the pots were as follows:

- 1- 2—Check.
- 3- 4—15 tons horse manure per acre.
- 5- 6—15 tons cow manure per acre.
- 7- 8—15 tons rotted manure per acre.
- 9-10—2½ tons oat straw per acre.
- 11-12— 3 tons corn stover per acre.
- 13-14— 2 tons timothy hay per acre.
- 15-16— 4 tons cowpea hay per acre.
- 17-18— 4 tons clover hay per acre.
- 19-20—Check.
- 21-22—15 tons horse manure per acre.
- 23-24—15 tons cow manure per acre.
- 25-26—15 tons rotted manure per acre.
- 27-28—2½ tons oat straw per acre.
- 29-30— 3 tons corn stover per acre.
- 31-32— 2 tons timothy hay per acre.
- 33-34— 4 tons cowpea hay per acre.
- 35-36— 4 tons clover hay per acre.

Pots 19 to 36 were seeded to oats and the others were kept bare to allow the taking of samples for bacteriological tests.

The rate of application of the materials used was based on farm conditions, approximately the same amounts being applied as if a maximum crop were grown and turned under in the soil or a heavy application of manure was made.

All of the materials were dried and ground before being applied, but the application of the manures was calculated on the wet basis while in all the other cases the dry basis was used. All applications were figured on the basis of two million pounds of soil per acre.

After mixing the various materials thoroughly with the soil, the oats were seeded in the proper pots and all received 100 c.c. of an infusion made by shaking for five minutes, fresh soil with water in the proportion of 100 gm. per 200 c.c. of water. This was to supply a vigorous bacterial flora from the soil in its natural state in order that the decomposition of the various materials might proceed as rapidly as it would in the field.

The optimum moisture content of the soil was determined and after the addition of the infusions sufficient additional water was supplied to bring the water content in each pot up to the optimum. The pots were then weighed and additions of water were made at regular intervals during the continuance of the experiment to maintain a constant weight.

The oats were harvested just prior to maturity and were dried, ground and analyzed.

Samples were drawn for bacteriological tests once every two or three weeks and the ammonifying, nitrifying, and azofying or nitrogen-fixing powers of the soils were determined.

The casein-fresh-soil method<sup>1</sup> and the dried blood-fresh-soil method were used for ammonification. The ammonium-sulfate-fresh soil method served for nitrification and the mannite-fresh-soil and dextrose-fresh-soil methods were employed for azofication.<sup>2</sup>

The samples for the bacteriological tests were drawn with the usual precautions to avoid contamination and thorough mixing was insured before the one-hundred-gram portions were weighed out for the various tests. The moisture content of all the soils was determined at each sampling and the moisture content of the soils in all the tests was adjusted to two-thirds of the saturation point.

In the nitrification tests the moisture content of the samples was kept up by additions of sterile water to weight every ten days.

The incubation took place at room temperature which was fairly constant at 23-25° C. The incubation period varied as will be noted in the later discussions.

The ammonification determinations were made in all cases except one by the magnesium-oxide method. In one instance the aeration method of Potter and Snyder<sup>3</sup> was used.

The nitrate determinations were made by the phenol-disulfonic acid method and total nitrogen was estimated by the regular Kjeldahl method.

#### THE EFFECT OF THE MATERIALS ADDED ON THE NITROGEN-CARBON RATIO IN THE SOIL.

The nitrogen and the carbon content of the soil and of all the materials used were determined and the nitrogen-carbon ratio calculated. These results are given in Table I.

TABLE I.  
NITROGEN AND CARBON IN SOIL AND IN MATERIALS USED.

Materials Analyzed.	Nitrogen Per Cent.	Carbon Per Cent.	Nitrogen-Carbon Ratio.
Soil .....	0.0988	1.3481	1 : 13.644
Horse manure .....	1.6468	38.7614	1 : 23.537
Cow manure .....	2.4176	36.6160	1 : 15.145
Rotted manure .....	2.4461	23.9047	1 : 9.772
Oat straw .....	.8590	38.1622	1 : 44.426
Corn stover .....	1.4762	39.8266	1 : 26.979
Timothy hay .....	.9727	38.1502	1 : 39.221
Cowpea hay .....	2.1852	42.1834	1 : 19.304
Clover hay .....	2.0564	41.3085	1 : 20.088

The soil used showed a satisfactorily wide ratio and hence the effects of the materials added cannot be expected to appear as definitely as might be the case did the soil itself contain a smaller amount of organic matter of a narrower ratio.

The rotted manure had the narrowest ratio of any of the materials employed and the oat straw the widest. The cow manure had a narrower ratio than the horse manure and the relative amounts of nitrogen and carbon in the legume hays were about the same as those in the horse manure.

In Table II are given the results showing the amounts of nitrogen and carbon added to the soils in the various materials applied and the nitrogen-carbon ratio in the soils after the applications were made.

It will be seen that all the materials applied brought about a widening of the nitrogen-carbon ratio except the rotted manure which narrowed the ratio. This is in accord with the results in the previous table which showed that the rotted manure had a narrower ratio than the soil itself and hence it might be expected to narrow the ratio in the soil. The oat straw widened the ratio more than any of the hays, particularly the legumes.

The horse manure brought about a greater widening of the ratio than the other materials applied, greater even than those which had a wider ratio than the horse manure. This is evidently due to the very much larger application of the horse manure than of the straw, stover and hays.

It will be recalled that the amounts of all the materials used were calculated as maximum field applications and hence it is interesting to note the relative influence of the different substances and to consider them from the field standpoint.

TABLE II.  
NITROGEN CARBON RATIO IN SOILS AFTER TREATMENT.

Pot No.	Treatment.	Materials added gm.	N added gm.	C added gm.	Total N gm.	Total C gm.	N-C Ratio.
1	Check . . . .	none	none	none	16.14	220.26	1 : 13.6
2	Check . . . . .	none	none	none	16.14	220.26	1 : 13.6
3	Horsemanure	78.19	1.29	30.31	17.43	250.57	1 : 14.4
4	Horsemanure	78.19	1.29	30.31	17.43	250.57	1 : 14.4
5	Cow manure	59.63	1.44	21.84	17.59	242.10	1 : 13.8
6	Cow manure	59.63	1.44	21.84	17.59	242.10	1 : 13.8
7	Rotted manure	83.65	2.05	20.00	18.19	240.26	1 : 13.2
8	Rotted manure	83.65	2.05	20.00	18.19	240.26	1 : 13.2
9	Oat straw . .	40.85	0.35	15.59	16.50	235.85	1 : 14.3
10	Oat straw . .	40.85	0.35	15.59	16.50	235.85	1 : 14.3
11	Corn stover .	49.02	0.72	19.52	16.87	239.79	1 : 14.2
12	Corn stover .	49.02	0.72	19.52	16.87	239.79	1 : 14.2
13	Timothy hay	32.68	0.32	12.47	16.46	232.73	1 : 14.1
14	Timothy hay	32.68	0.32	12.47	16.46	232.73	1 : 14.1
15	Cowpea hay .	65.36	1.43	27.57	17.57	247.83	1 : 14.1
16	Cowpea hay .	65.36	1.43	27.57	17.57	247.83	1 : 14.1
17	Clover hay . .	65.36	1.34	27.00	17.49	247.26	1 : 14.1
18	Clover hay . .	65.36	1.34	27.00	17.49	247.26	1 : 14.1

Rotted manure actually narrowed the ratio and hence might be considered as having the least effect on the decomposition processes, while

all the other materials increased the proportion of carbon to nitrogen and hence should increase bacterial activities to a much greater extent.

Among the straws and hays used, with the exception of timothy hay, the wider the nitrogen-carbon ratio, the greater the widening of the ratio in the soil when they were applied. It might be expected, therefore, that the materials of the wider ratios would give greater effects on bacterial processes than those whose content in nitrogen and carbon was more nearly the same.

The changes in the nitrogen-carbon ratio in this soil, by the applications of these materials were very much smaller, undoubtedly, than would have occurred if a soil of a narrower ratio had been chosen.

It is apparent, however, that the ordinary humus-forming materials on the farm, when applied to the soil, widen the nitrogen-carbon ratio of the soil even when it is not extremely narrow and hence should be expected to increase bacterial activities to a beneficial extent, and consequently increase decomposition processes and the production of available plant food, and increase also the fixation of nitrogen from the atmosphere.

#### THE AMMONIFICATION EXPERIMENTS.

The experiment was started on December 5th and the first sampling was made on December 24th, in order to allow time for decomposition to commence. Samplings were made approximately every two weeks, the dates being January 7, January 28, February 12, February 26 and March 12.

The results of the ammonification tests in which casein was used and those secured with dried blood are considered separately, but general conclusions will be drawn from both lots of experiments. The incubation period in the case of casein was three and four days, while with the dried blood it was six and seven days.

#### THE AMMONIFICATION OF CASEIN.

The results in a summarized form, of the ammonification experiments with casein are given in Table III and in Table IV. Where individual results were widely at variance with the general trend of the results as shown throughout the six samplings, they were omitted from the averages. The tests at the first three samplings were incubated four days and those on the remaining dates were incubated three days.

Very much larger amounts of ammonia were produced in the samples taken on January 7, than in the tests at the other dates. This is probably due to an increase in room temperature to 29° C. which occurred while the samples were being incubated.

It will be seen in Table III that for the most part the duplicate determinations agreed very satisfactorily, or at least much better than in the case of other tests with different nitrogenous materials.

TABLE III.  
THE AMMONIFICATION OF CASEIN.

Pot No.	Lab. No.	I. December 24.		II. January 7.		III. January 28.	
		Ammonia Mg. N.	Average Mg. N.	Ammonia Mg. N.	Average Mg. N.	Ammonia Mg. N.	Average Mg. N.
1	1	82.32	87.54	100.94	102.84	85.63	85.63
2	2	92.47	87.54	104.72	102.84	89.29*	85.63
3	3	87.06	89.01	101.85	102.62	83.67	83.67
4	4	90.97	89.01	103.40	102.62	89.15*	83.67
5	5	90.29	90.63	105.57	105.14	92.05	93.13
6	6	90.97	90.63	104.72	105.14	94.22	93.13
7	7	89.76	89.76	103.83	104.16	90.28	88.45
8	8	84.05*	89.76	104.49	104.16	86.61	88.45
9	9	86.08*	89.01	105.57	107.12	86.19	88.09
10	10	89.01	89.01	108.68	107.12	89.99	88.09
11	11	88.49	89.24	104.72	104.60	81.83*	85.73
12	12	89.99	89.24	104.49	104.60	85.73	85.73
13	13	88.49	88.75	103.83	104.82	90.17	88.74
14	14	89.01	88.75	105.81	104.82	87.31	88.74
15	15	89.01	89.99	102.93	106.02	85.63	85.06
16	16	90.97	89.99	109.11	106.02	84.50	85.06
17	17	86.53	88.75	106.04	105.71	85.63	84.50
18	18	90.97	88.75	105.38	105.71	83.38	84.50
19	19	86.51	88.90	98.79	99.77	81.96	82.81
20	20	90.29	88.90	100.76	99.77	83.67	82.81
21	21	89.01	89.99	102.51	101.63	82.25	85.56
22	22	90.97	89.99	100.76	101.63	88.87	85.56
23	23	89.76	90.36	105.10	103.80	84.80	87.12
24	24	90.97	90.36	102.51	103.80	89.43	87.12
25	25	92.17	90.25	102.32	103.71	86.61	84.52
26	26	88.14	90.25	105.10	103.71	82.53	84.52
27	27	89.54	89.01	104.25	100.97	84.38	82.55
28	28	88.49	89.01	97.70	100.97	78.73	82.55
29	29	85.55	85.55	105.10	102.25	78.59	78.57
30	30	85.55	85.55	99.21	102.25	78.55	78.57
31	31	89.54	89.54	105.51	106.67	78.59	79.36
32	32	89.54	89.54	107.83	106.67	80.14	79.36
33	33	90.97	90.48	108.03	107.13	86.33	86.75
34	34	89.99	90.48	106.23	107.13	87.18	86.75
35	35	90.97	89.01	105.57	106.35	86.47	86.47
36	36	87.06	89.01	107.13	106.35	81.83*	86.47

Pot No.	Lab. No.	IV. February 12.		V. February 26.		VI. March 12.	
		Ammonia Mg. N.	Average Mg. N.	Ammonia Mg. N.	Average Mg. N.	Ammonia Mg. N.	Average Mg. N.
1	1	84.38	85.26	88.69	89.58	92.39	93.40
2	2	86.15	85.26	90.47	89.58	94.42	93.40
3	3	83.66	84.82	88.58	89.47	91.44	93.05
4	4	85.99	84.82	90.37	89.47	94.69	93.05
5	5	87.04	86.51	92.75	92.45	96.31	95.90
6	6	85.99	86.51	92.16	92.45	95.50	95.90
7	7	89.89	87.58	92.75	92.75	98.48	96.45
8	8	85.27	87.58	lost	92.75	94.42	96.45
9	9	84.91	86.07	96.03	94.70	98.88	96.38
10	10	87.24	86.07	93.38	94.70	93.88	96.38
11	11	85.09	85.18	92.75	92.75	98.34	96.38
12	12	85.27	85.18	92.75	92.75	94.42	96.38
13	13	85.09	85.98	91.26	92.00	94.01	94.01
14	14	86.87	85.98	92.75	92.00	86.02*	94.01
15	15	86.51	87.05	92.45	92.75	94.28	93.81
16	16	87.60	87.05	93.05	92.75	93.34	93.81
17	17	85.79	84.10	91.86	92.62	93.74	94.14
18	18	82.41	84.10	93.38	92.62	94.55	94.14
19	19	82.60	82.60	92.45	93.49	92.25	90.42
20	20	82.60	82.60	94.54	93.49	88.59	90.42
21	21	87.42	86.25	87.98	89.22	92.93	93.74
22	22	85.09	86.25	90.47	89.22	94.55	93.74
23	23	83.49	85.10	88.47	89.57	92.79	92.79
24	24	86.71	85.10	90.67	89.57	88.32*	92.79
25	25	86.87	85.44	89.18	88.88	90.90	90.35
26	26	84.02	85.44	88.58	88.88	89.81	90.35
27	27	86.71	85.62	92.45	92.30	93.20	91.23
28	28	84.54	85.62	92.16	92.30	89.27	91.23
29	29	85.27	84.81	91.56	90.01	91.72	93.88
30	30	84.45	84.81	88.47	90.01	96.04	93.88
31	31	85.99	87.14	91.26	91.26	87.78	89.61
32	32	88.29	87.14	lost	91.26	91.44	89.61
33	33	87.76	87.68	91.26	89.92	93.61	92.59
34	34	87.60	87.68	88.58	89.92	91.57	92.59
35	35	87.60	85.78	88.58	88.52	93.88	93.20
36	36	85.99	85.78	88.47	88.52	92.52	93.20

\*Results omitted from the averages.

The differences in ammonifying power between the different soils were not, however, very large, and it is very difficult in such cases to draw definite conclusions.

TABLE IV.  
THE AMMONIFICATION OF CASEIN.

Pot No.	Treatment.	I.		II.		III.	
		Ammonia Mg. N.	Average Mg. N.	Ammonia Mg. N.	Average Mg. N.	Ammonia Mg. N.	Average Mg. N.
1	Check .....	87.54	.....	102.84	.....	85.63	.....
2	Check .....	89.01	88.27	102.62	102.73	83.67	84.65
3	Horse manure .....	90.63	.....	105.14	.....	93.13	.....
4	Horse manure .....	89.76	90.19	104.16	104.65	88.45	90.79
5	Cow manure .....	89.01	.....	107.12	.....	88.09	.....
6	Cow manure .....	89.24	88.39	104.60	105.86	85.73	86.91
7	Rotted manure .....	88.75	.....	104.82	.....	88.74	.....
8	Rotted manure .....	89.99	89.37	106.02	105.42	85.06	86.90
9	Oat straw .....	88.75	.....	105.71	.....	84.50	.....
10	Oat straw .....	88.90	88.82	99.77	102.74	82.81	83.65
11	Corn stover .....	89.99	.....	101.63	.....	85.56	.....
12	Corn stover .....	90.36	90.17	103.80	102.71	87.12	86.34
13	Timothy hay .....	90.25	.....	103.71	.....	84.52	.....
14	Timothy hay .....	89.01	89.63	100.97	102.34	82.55	83.53
15	Cowpea hay .....	85.55	.....	102.25	.....	78.57	.....
16	Cowpea hay .....	89.54	87.54	106.67	104.46	79.36	78.96
17	Clover hay .....	90.48	.....	107.13	.....	86.75	.....
18	Clover hay .....	89.01	89.74	106.35	106.74	86.47	86.61

Pot No.	Treatment.	IV.		V.		VI.	
		Ammonia Mg. N.	Average Mg. N.	Ammonia Mg. N.	Average Mg. N.	Ammonia Mg. N.	Average Mg. N.
1	Check .....	85.26	.....	89.58	.....	93.40	.....
2	Check .....	84.82	85.04	89.47	89.52	93.05	93.22
3	Horse manure .....	86.51	.....	92.45	.....	95.90	.....
4	Horse manure .....	87.58	86.54	92.75	92.60	96.45	96.17
5	Cow manure .....	86.07	.....	94.70	.....	96.38	.....
6	Cow manure .....	85.18	85.62	92.75	93.72	96.38	96.38
7	Rotted manure .....	85.98	.....	92.00	.....	94.01	.....
8	Rotted manure .....	87.05	86.56	92.75	92.35	92.79	93.91
9	Oat straw .....	84.10	.....	92.62	.....	94.14	.....
10	Oat straw .....	82.60	83.35	93.49	93.05	90.42	92.28
11	Corn stover .....	86.25	.....	89.22	.....	93.74	.....
12	Corn stover .....	85.10	85.67	89.57	89.38	92.79	93.26
13	Timothy hay .....	85.44	.....	88.88	.....	90.35	.....
14	Timothy hay .....	85.62	85.53	92.30	90.39	91.23	90.79
15	Cowpea hay .....	84.81	.....	90.01	.....	93.88	.....
16	Cowpea hay .....	87.14	85.97	91.26	90.63	89.61	91.74
17	Clover hay .....	87.68	.....	89.92	.....	92.59	.....
18	Clover hay .....	85.78	86.73	88.52	89.22	93.20	92.89

In general, however, considering the results as a whole, it appears from Table IV that horse manure, cow manure, and rotted manure favored the ammonifying bacteria to the greatest extent. Next in order came clover hay, corn stover, oat straw, cowpea hay and timothy hay, respectively. In the case of the latter materials the differences were not large, and their relative effects varied greatly at the different samplings.

For the most part, however, all the materials increased the ammonifying power of the soil, according to the tests and while in a few instances some depressing action was noted, the figures were not widely enough separated for the results to be conclusive.

Some depression in the ammonifying power of soils may occur immediately following the application of materials similar to those used in these experiments, but after such substances commence to decompose,

any decrease in the activities of the ammonifying bacteria would hardly be expected. Some decomposition of all the materials used in this work had undoubtedly occurred prior to the making of any tests and hence it seems probable that the slight depressions noted should be considered merely as indications of the absence of any particular increasing action of the substances applied. The variations from the results with the check soils should in such a case be considered as due to experimental error or accidental contamination. Much more distinctive results than those secured here must be obtained before the occurrence of any depressing action could be considered as the rule with the use of these materials.

In short, it seems safe to conclude that applications of humus-forming materials increased the ammonifying power of soils as indicated by tests with the casein-fresh-soil method. The manures had a greater effect than straw, stover or hays; and horse manure and cow manure showed much more influence than rotted manure. It must be recalled here that the bacterial factor was the same in all the pots, as the materials were all added in a dry condition and different effects were due, therefore, to differences in amounts added or in composition.

While the casein-fresh-soil method gives very satisfactory results from the standpoint of agreement of duplicates and because of ease of manipulation, it is apparent that some further modification will be necessary to make it possible for distinctive results to be secured with its use. The dried-blood-fresh-soil method although much more difficult to use is evidently better suited for general soil studies and causes a wider difference to be shown in ammonifying power between soils differently treated.

Reference will again be made to the results with casein after the dried-blood experiments are considered.

#### THE AMMONIFICATION OF DRIED BLOOD.

The samples drawn on the same dates as previously mentioned when the ammonifying power of the soils was tested with casein, were used for ammonification tests with dried blood, except that no tests were made on January 7. An additional sampling was made, however, on March 24, so that six series of results were secured here. Thus there was provided a comparison of the two methods as well as additional data on the ammonifying power of the soils.

The results of the tests with dried blood are given in Table V and the summarized results appear in Table VI.

The incubation period for the first, third and fifth sampling was seven days; for the fourth and sixth it was six days and in the second series one-half of the determinations were made on the fifth day and the duplicate half on the sixth day. This second series was distilled by the aera-



maximum field applications had little or no effect on the influence exerted by these substances on ammonification in *this particular soil*. The different effects were probably due to the variations in chemical composition of the materials used.

Comparing the results of the ammonification tests as a whole, using the casein and the dried blood methods, it is apparent that the latter allows of much greater differentiation between the ammonifying power of soils differently treated. The casein method permits of the securing of much better agreement among duplicate determinations, but this point is of minor importance to the securing of results distinguishing more widely between ammonification in different soils. Some further modification in the technique of the casein method may remedy the difficulty mentioned, but until such a change is made the dried blood method must be considered the more satisfactory.

#### THE NITRIFICATION EXPERIMENTS.

To determine the effect of the various materials used in this work on the nitrifying power of the soil, samples secured on the dates previously mentioned were tested by the ammonium-sulfate-fresh-soil method as has been described. The tests on February 12, were incubated for 27 days and all the other tests were made in 28 days.

The results of the determinations are given in Tables VII, and the average results appear in Table VIII.

A few of the results are omitted from the averages because of evident abnormality. It will be noted, however, that as a whole the duplicate determinations agreed very satisfactorily.

Considering now the results given in Table VIII, it appears that the differences in nitrifying power were not pronounced. In general it seems, however, that the cowpea hay and clover hay had the greatest action on the nitrifiers and the manures a lesser effect, while the straws, stover and timothy hay showed little influence on nitrification. The differences were too slight to warrant definite conclusions in the case of the three latter materials, and hence the only statement which can be made is that these materials exerted practically no influence on nitrification. The small variations in the nitrifying power of the soils used in this work might have been increased by a longer incubation period. It seems that possibly larger differences might have been found with variations in the method employed, but from the standpoint of these experiments it is apparent that the nitrogen-carbon ratio of the materials used had no effect on the influence of the substances on the nitrifying power of the soils. The effects of the materials were apparently exerted on nitrification regardless of the ratio of nitrogen to carbon in them and dependent probably as in the case of ammonification on the chemical compounds present in the materials.

TABLE VII.  
THE NITRIFICATION OF AMMONIUM SULFATE.

Pot No.	Lab. No.	I. December 24.		II. January 7.		III. January 28.	
		Nitrate Mg. N.	Average Mg. N.	Nitrate Mg. N.	Average Mg. N.	Nitrate Mg. N.	Average Mg. N.
1	1	20.41	.....	19.74	.....	17.04	.....
2	2	20.83	20.62	19.99	19.36	17.04	17.04
3	3	20.83	.....	18.99	.....	17.04	.....
4	4	20.83	20.83	19.74	19.36	16.85	16.94
5	5	20.83	.....	20.00	.....	18.99	.....
6	6	21.23	21.53	19.74	19.87	18.99	18.99
7	7	21.73	.....	20.00	.....	18.52	.....
8	8	21.23	21.48	19.59	19.87	16.85	17.68
9	9	22.22	.....	22.06	.....	16.48	.....
10	10	22.72	22.46	22.40	22.23	16.48	16.48
11	11	20.41	.....	23.81	.....	18.52	.....
12	12	21.73	21.07	24.19	24.00	16.48	17.50
13	13	21.73	.....	22.73	.....	15.96	.....
14	14	21.73	21.73	22.39	22.56	16.85	16.90
15	15	22.22	.....	21.74	.....	16.85	.....
16	16	22.72	22.47	22.40	22.07	17.04	16.94
17	17	20.83	.....	21.13	.....	17.04	.....
18	18	21.23	21.03	20.83	20.98	16.85	16.94
19	19	21.23	.....	21.13	.....	17.04	.....
20	20	20.83	21.03	21.13	21.13	16.85	16.94
21	21	20.83	.....	20.27	.....	17.65	.....
22	22	21.23	21.03	20.48	20.37	16.99	17.32
23	23	21.23	.....	18.75	.....	18.07	.....
24	24	20.83	21.03	18.99	18.87	15.96	17.01
25	25	20.41	.....	22.06	.....	16.85	.....
26	26	20.83	20.62	21.74	21.90	17.04	16.94
27	27	20.83	.....	22.06	.....	16.66	.....
28	28	20.83	20.83	21.74	21.90	17.44	17.05
29	29	21.23	.....	25.64	.....	18.99	.....
30	30	21.73	21.48	24.59	25.61	18.75	18.87
31	31	21.23	.....	25.86	.....	19.48	.....
32	32	21.23	21.23	25.42	25.64	18.52	19.00
33	33	21.23	.....	23.81	.....	18.52	.....
34	34	21.73	21.48	23.40	23.60	17.04	17.78
35	35	21.23	.....	23.40	.....	18.52	.....
36	36	22.22	21.72	23.40	23.40	17.65	18.08

Pot No.	Lab. No.	IV. February 12.		V. February 26.		VI. March 12	
		Nitrate Mg. N.	Average Mg. N.	Nitrate Mg. N.	Average Mg. N.	Nitrate Mg. N.	Average Mg. N.
1	1	20.83	.....	22.06	.....	24.19	.....
2	2	21.43	21.13	21.74	21.90	23.40	23.79
3	3	19.74	.....	20.27	.....	24.57	.....
4	4	21.43	20.58	22.06	21.16	23.40	23.98
5	5	18.75	.....	22.06	.....	28.47	.....
6	6	19.59	19.17	21.74	21.90	22.40	25.43
7	7	20.00	.....	22.06	.....	22.40	.....
8	8	19.74	19.87	22.06	22.06	25.86	24.13
9	9	22.39	.....	22.06	.....	25.86	.....
10	10	21.43	21.91	22.39	22.22	27.77	26.81
11	11	19.23	.....	22.73	.....	25.42	.....
12	12	21.74	20.43	21.43	22.08	27.77	26.59
13	13	22.73	.....	22.40	.....	26.31	.....
14	14	23.40	23.06	24.57	23.48	23.40	24.85
15	15	20.27	.....	21.74	.....	27.77	.....
16	16	20.27	20.27	24.57	23.15	24.19	25.98
17	17	20.55	.....	22.73	.....	23.81	.....
18	18	20.55	20.55	19.74	21.23	22.40	23.10
19	19	18.07*	.....	22.06	.....	26.31	.....
20	20	17.65*	.....	18.75*	22.06	23.81	25.06
21	21	18.99	.....	22.06	.....	25.42	.....
22	22	20.83	19.91	20.27	21.16	26.31	25.86
23	23	20.27	.....	22.06	.....	25.42	.....
24	24	22.06	21.16	19.23*	22.06	22.40	23.91
25	25	18.07*	.....	20.00	.....	25.42	.....
26	26	20.27	20.27	20.27	20.13	23.81	24.61
27	27	19.74*	.....	22.06	.....	25.00	.....
28	28	21.43	21.43	21.74	21.90	24.57	24.78
29	29	22.73	.....	22.73	.....	27.27	.....
30	30	22.40	22.56	23.81	23.27	25.42	26.34
31	31	22.40	.....	24.19	.....	27.27	.....
32	32	23.81	23.10	23.40	23.79	25.86	26.56
33	33	20.55	.....	22.39	.....	27.77	.....
34	34	22.73	21.64	20.55	21.47	25.42	26.59
35	35	23.40	.....	24.19	.....	26.78	.....
36	36	20.00	21.70	20.83	22.51	27.77	27.27

\*Results omitted from the averages.

TABLE VIII.  
THE NITRIFICATION OF AMMONIUM SULFATE.

Pot No.	Treatment.	I.		II.		III.	
		Average Mg. N.	Nitrate Mg. N.	Average Mg. N.	Nitrate Mg. N.	Nitrate Mg. N.	Average Mg. N.
1	Check .....	20.62		19.36		17.04	
2	Check .....	20.83	20.72	19.36	19.36	16.94	16.99
3	Horse manure .....	21.53		19.87		18.99	
4	Horse manure .....	21.48	21.50	19.78	19.82	17.68	18.33
5	Cow manure .....	22.46		22.23		16.48	
6	Cow manure .....	21.07	21.76	24.00	23.11	17.50	16.99
7	Rotted manure .....	21.73		22.56		16.90	
8	Rotted manure .....	22.47	22.10	22.07	22.31	16.94	16.92
9	Oat straw .....	21.03		20.98		16.94	
10	Oat straw .....	21.03	21.03	21.13	21.05	16.94	16.94
11	Corn stover .....	21.03		20.37		17.32	
12	Corn stover .....	21.03	21.03	18.87	19.62	17.01	17.16
13	Timothy hay .....	20.62		21.90		16.94	
14	Timothy hay .....	20.83	20.72	21.90	21.90	17.05	16.99
15	Cowpea hay .....	21.48		25.61		18.87	
16	Cowpea hay .....	21.23	21.35	25.64	25.62	19.00	18.93
17	Clover hay .....	21.48		23.60		17.78	
18	Clover hay .....	21.72	21.60	23.40	23.50	18.08	17.93

Pot No.	Treatment.	IV.		V.		VI.	
		Nitrate Mg. N.	Average Mg. N.	Nitrate Mg. N.	Average Mg. N.	Nitrate Mg. N.	Average Mg. N.
1	Check .....	21.13		21.90		23.79	
2	Check .....	20.58	20.85	21.16	21.53	23.98	23.88
3	Horse manure .....	19.17		21.90		25.43	
4	Horse manure .....	19.87	19.52	22.06	21.98	24.13	24.78
5	Cow manure .....	21.91		22.22		26.81	
6	Cow manure .....	20.43	21.17	22.08	22.15	26.59	26.70
7	Rotted manure .....	23.06		23.48		24.85	
8	Rotted manure .....	20.27	21.66	23.15	23.31	25.98	25.91
9	Oat straw .....	20.55		21.23		23.10	
10	Oat straw .....		20.55	22.06	21.64	25.06	24.08
11	Corn stover .....	19.91		21.16		25.86	
12	Corn stover .....	21.16	20.53	22.06	21.61	23.91	24.88
13	Timothy hay .....	20.27		20.13		24.61	
14	Timothy hay .....	21.43	20.85	21.90	21.01	24.78	24.69
15	Cowpea hay .....	22.56		23.27		26.34	
16	Cowpea hay .....	23.10	22.83	23.79	23.53	26.56	26.45
17	Clover hay .....	21.64		21.47		26.59	
18	Clover hay .....	21.70	21.67	22.51	21.99	27.27	26.93

It is interesting to note that the manure which exerted the greatest effect on ammonification showed also comparatively large effects on nitrification, while the legume hays which showed lesser effects on ammonification than the manures gave a greater influence on nitrification. Just why this should be the case is difficult to determine as ordinarily materials which favor ammonification in field soils will favor also nitrification, unless the amounts of organic matter added are so large that nitrification is entirely inhibited. It seems to be a matter of considerable doubt at present whether it is possible to add sufficient organic matter to field soils to prevent nitrification. However that may be, it is apparent here that nitrification was not restricted by any of the maximum applications of the common materials used and ammonification was increased as described, hence it might be expected that the effects would be in the same direction for both processes. It is possible, however, that different materials might increase both processes, but to different degrees.

It is important to note, however, from these results that the common humus-forming materials, such as are used on the farm when applied in

maximum amounts did not depress the nitrifying power of the soil, at least of this particular soil. On the other hand, there was an increase in nitrification to a more or less pronounced extent with the different materials. In the case of soils containing more organic matter or material of a narrower nitrogen-carbon ratio, it is difficult to predict the effect, but inasmuch as organic matter in such large amounts as were used here, particularly in the case of the leguminous green manures, would not be used unless the soils were low in nitrogen, it seems safe to say that there is no danger of restricting nitrification in soils by additions of amounts of organic matter such as would be used in the field.

In general from these experiments it is apparent that nitrification was increased by additions of organic materials such as are made on the farm, and these increases were independent of the nitrogen-carbon ratio in the materials, although there were some indications that the materials of a narrower ratio gave a greater effect than those of a wider ratio, but the results were not conclusive. Inasmuch as the latter possibility is the opposite of the case with ammonification, it is apparent that more definite results must be secured before any conclusions should be drawn.

#### THE AZOFICATION EXPERIMENTS.

The samples drawn on the six dates mentioned previously were tested for their azofying or nitrogen-fixing power by the fresh-soil method. At the first sampling, mannite (5 gm. per 100 gm. of soil) was used and at the later dates dextrose was employed, being added from solution at the rate of 3 gm. per 100 gm. of soil.

The incubation period was eleven days, except in the case of the second sampling when the tests were allowed to incubate fourteen days.

The complete results of the tests are given in Table IX and the summarized results appear in Table X.

As might be expected, there were considerable variations in the results of the duplicate determinations. The method used for the determination of total nitrogen does not permit of the estimation of such small amounts of nitrogen as sometimes represent the nitrogen fixation. In some instances a smaller amount of nitrogen was actually found after the incubation period, but it was hardly possible for any loss of nitrogen to occur and hence such results should be attributed to the fact that the method is not accurate for small amounts of nitrogen. These low results are eliminated from the averages and are interpreted merely as representing, therefore, the absence of any azofication.

In calculating the results, the total nitrogen present in the soils in the tests before incubation was estimated and the nitrogen present at the end subtracted to determine the amount of nitrogen fixed. A slight error is, of course, introduced here in case not all of the mannite or dextrose added was used by the bacteria when the unused portion would be included

TABLE IX.  
AZOFICATION.

			I. December 24.			II. January 7.		
Pot No.	Lab. No.	N. in Orig. Soil Mg.	N. after incub. Mg.	N. fixed Mg.	Average Mg.	N. after incub. Mg.	N. fixed Mg.	Average Mg.
1	1	98.80	99.50	.70	.....	110.10	11.30	.....
..	2	98.80	98.80	.00	.35	110.20	1.40	6.35
2	3	98.80	102.30	3.50	.....	104.40	5.60	.....
..	4	98.80	100.90	2.10	2.80	105.80	7.00	6.30
3	5	106.56	110.80	4.24	.....	115.70	9.14	.....
..	6	106.56	107.20	.64	2.44	115.70	9.14	9.14
4	7	106.56	107.20	.64	.....	115.00	8.44	.....
..	8	106.56	105.10	-1.46*	.64	115.70	9.14	8.79
5	9	107.62	110.10	2.48	.....	119.90	12.28	.....
..	10	107.62	111.50	3.88	3.18	115.00	7.38	9.83
6	11	107.62	110.10	2.48	.....	118.50	10.88	.....
..	12	107.62	111.50	3.88	3.18	115.70	8.08	9.48
7	13	111.32	111.50	.18	.....	117.80	6.48	.....
..	14	111.32	111.50	.18	.18	122.80	11.48	8.98
8	15	111.32	105.80	-5.52*	.....	119.90	8.58	.....
..	16	111.32	113.60	2.28	2.28	121.40	10.08	9.33
9	17	100.97	104.40	3.43	.....	105.80	4.83	.....
..	18	100.97	107.20	6.23	3.83	104.40	3.43	4.13
10	19	100.97	100.20	-.77*	.....	112.90	11.93	.....
..	20	100.97	101.50	.53	.53	108.60	7.63	9.78
11	21	103.23	105.80	2.57	.....	109.40	6.17	.....
..	22	103.23	107.90	4.67	3.62	111.50	8.27	7.22
12	23	103.23	100.90	2.33	.....	108.60	5.37	.....
..	24	103.23	106.50	3.27	2.80	111.50	8.27	6.82
13	25	100.75	103.00	2.25	.....	105.80	5.05	.....
..	26	100.75	105.10	4.35	3.30	114.30	13.55	9.30
14	27	100.75	102.30	1.55	.....	108.60	7.85	.....
..	28	100.75	107.20	6.45	4.00	109.40	8.65	8.25
15	29	107.55	108.60	1.05	.....	112.20	4.65	.....
..	30	107.55	107.20	-.35*	1.05	112.90	5.35	5.00
16	31	107.55	107.20	-.35*	.....	111.50	3.95	.....
..	32	107.55	107.20	-.35*	.....	112.20	4.65	4.30
17	33	107.03	110.10	3.07	.....	111.50	4.47	.....
..	34	107.03	103.00	-4.03*	3.07	108.60	1.57	3.02
18	35	107.03	108.00	.97	.....	112.90	5.87	.....
..	36	107.03	107.20	.17	.57	115.70	4.47	5.17

## III.

January 28.

## IV.

February 12.

Pot No.	Lab. No.	N. in Orig. Soil Mg.	N. after incub. Mg.	N. fixed Mg.	Average Mg.	N. after incub. Mg.	N. fixed Mg.	Average Mg.
1	1	98.80	102.30	3.50	.....	102.30	3.50	.....
..	2	98.80	111.50	12.70	8.10	100.90	2.10	2.80
2	3	98.80	105.80	7.00	.....	98.80	.00	.....
..	4	98.80	101.40	5.60	6.30	107.20	8.40	4.20
3	5	106.56	122.10	15.54	.....	110.80	4.24	.....
..	6	106.56	116.40	9.84	12.69	107.90	1.34	2.79
4	7	106.56	116.40	9.84	.....	112.20	5.64	.....
..	8	106.56	115.70	9.14	9.49	109.40	2.84	4.24
5	9	107.62	121.30	13.68	.....	111.50	3.88	.....
..	10	107.62	117.10	9.48	11.58	117.80	10.18	7.03
6	11	107.62	108.20	-.42*	.....	115.00	7.38	.....
..	12	107.62	119.20	11.58	11.58	111.50	3.88	5.63
7	13	111.32	117.10	5.78	.....	115.70	4.38	.....
..	14	111.32	120.60	9.28	7.53	112.90	1.58	2.98
8	15	111.32	122.80	11.48	.....	121.30	9.98	.....
..	16	111.32	131.20	19.88	10.68	115.70	4.38	7.18
9	17	100.97	112.20	11.23	.....	115.70	14.73	.....
..	18	100.97	112.90	11.93	11.58	111.50	10.53	12.63
10	19	100.97	117.10	16.13	.....	104.40	3.43	.....
..	20	100.97	112.90	11.93	14.03	107.20	6.23	4.83
11	21	103.23	107.20	3.97	.....	108.60	5.37	.....
..	22	103.23	105.80	2.57	3.27	104.40	1.17	3.27
12	23	103.23	111.50	8.27	.....	112.90	9.67	.....
..	24	103.23	109.40	6.17	7.22	107.90	4.67	7.17
13	25	100.75	105.80	5.05	.....	104.40	3.65	.....
..	26	100.75	104.40	3.65	4.35	108.60	7.85	5.75
14	27	100.75	103.00	2.25	.....	106.50	5.75	.....
..	28	100.75	108.60	7.85	5.05	104.40	3.65	4.70
15	29	107.55	112.90	5.35	.....	110.10	2.55	.....
..	30	107.55	115.00	7.45	6.40	105.10	-2.45*	2.55
16	31	107.55	116.40	8.85	.....	112.90	5.35	.....
..	32	107.55	112.20	4.65	6.75	112.20	4.65	5.00
17	33	107.03	113.60	6.57	.....	110.80	3.77	.....
..	34	107.03	114.30	7.27	6.92	108.60	1.57	2.67
18	35	107.03	108.60	1.57	.....	112.90	5.87	.....
..	36	107.03	111.50	4.47	3.02	110.10	3.07	4.47

\*Results omitted from the averages.

TABLE IX. (Continued).

Pot No.	Lab. No.	V. February 26.				VI. March 12.		
		N. in Orig. Soil Mg.	N. after incub. Mg.	N. fixed Mg.	Average Mg.	N. after incub. Mg.	N. fixed Mg.	Average Mg.
1	1	98.80	103.70	4.90	.....	106.50	7.70	.....
..	2	98.80	100.90	2.10	3.50	105.10	6.30	7.00
2	3	98.80	103.70	4.90	.....	105.80	7.00	.....
..	4	98.80	103.00	4.20	4.55	107.20	8.40	7.70
3	5	106.56	108.60	2.04	.....	117.10	10.54	.....
..	6	106.56	110.10	3.54	2.79	118.50	11.94	11.24
4	7	106.56	107.20	.66	.....	115.00	8.44	.....
..	8	106.56	115.70	9.14	4.70	118.50	11.94	10.19
5	9	107.62	111.50	3.88	.....	118.50	10.88	.....
..	10	107.62	119.90	12.28	8.08	125.60	17.98	14.43
6	11	107.62	119.90	12.28	.....	120.60	12.98	.....
..	12	107.62	112.90	5.28	8.78	115.70	8.08	10.53
7	13	111.32	115.00	3.68	.....	115.00	3.68	.....
..	14	111.32	115.70	4.38	4.03	127.70	16.38	10.03
8	15	111.32	113.60	2.28	.....	122.80	11.48	.....
..	16	111.32	118.50	7.18	4.73	127.00	15.68	13.58
9	17	100.97	108.60	7.63	.....	106.50	5.53	.....
..	18	100.97	108.60	7.63	7.63	108.60	7.63	6.58
10	19	100.97	105.10	4.23	.....	104.40	3.43	.....
..	20	100.97	111.50	10.53	7.38	112.90	11.93	7.66
11	21	103.23	107.20	3.97	.....	111.50	8.27	.....
..	22	103.23	110.10	6.87	5.42	106.50	3.27	5.77
12	23	103.23	107.90	4.67	.....	109.40	6.17	.....
..	24	103.23	110.80	7.57	6.12	110.10	6.87	6.52
13	25	100.75	107.20	6.45	.....	105.80	5.05	.....
..	26	100.75	104.40	3.65	5.05	112.90	12.15	8.60
14	27	100.75	107.20	6.45	.....	110.10	9.35	.....
..	28	100.75	112.90	12.15	9.30	109.40	8.65	9.00
15	29	107.55	113.60	6.05	.....	108.60	1.05	.....
..	30	107.55	107.20	— .35*	6.05	110.10	2.55	1.80
16	31	107.55	110.10	2.55	.....	108.60	1.05	.....
..	32	107.55	107.90	.35	1.45	112.20	4.65	2.85
17	33	107.03	110.10	3.07	.....	109.40	2.37	.....
..	34	107.03	111.50	4.47	3.77	110.10	3.07	2.72
18	35	107.03	110.10	3.07	.....	112.20	5.17	.....
..	36	107.03	111.50	4.47	3.77	109.40	2.37	3.77

\*Results omitted from the averages.

in the sample analyzed after incubation. In such a case the results would be slightly lower than they should be, hence the amounts of nitrogen fixed from the atmosphere may be too low, but that fact need not interfere with the interpretation of the results.

Considering the results given in Table X, it is apparent that the addition of various organic materials to the soil influenced to a considerable extent the fixation of nitrogen by non-symbiotic bacteria. In some cases the amount of nitrogen fixed in the eleven-day incubation period amounted to one-sixth of the nitrogen originally present in the soil.

The soils receiving cow manure and oat straw showed for the most part the greatest increase in azofying power and the influence of the rotted manure was only slightly less than that of the cow manure.

The horse manure gave less effect than the other manures and about the same in most cases as the timothy hay. The corn stover also affected the azofying power of the soil to about the same extent as the horse manure.

The cowpea hay and the clover hay exerted the smallest effect of any of the materials on the azofying power of the soil.

It appears, therefore, from the results as a whole that the nitrogen-carbon ratio of the various humus-forming materials applied to the soil was of little significance from the standpoint of the effect on azofication. The influence of the materials was exerted regardless of the nitrogen-carbon ratio. Thus the oat straw of a wide ratio and the cow manure of a narrower ratio had about the same effect. Similarly the timothy hay and the horse manure of wide and narrow ratios respectively had considerable influence. Again the rotted manure of a very narrow ratio exerted as much effect on azofication as the timothy hay which had a wide ratio.

TABLE X.  
AZOFICATION.

Pot No.	Treatment.	I.		II.		III.	
		N. fixed Mg.	Average Mg.	N. fixed Mg.	Average Mg.	N. fixed Mg.	Average Mg.
1	Check	.35	.....	6.35	.....	8.10	.....
2	Check	2.80	1.57	6.30	6.32	6.30	7.20
3	Horse manure	2.44	.....	9.14	.....	12.69	.....
4	Horse manure	.64	1.54	8.79	8.96	9.49	11.09
5	Cow manure	3.18	.....	9.83	.....	11.58	.....
6	Cow manure	3.18	3.18	9.48	9.65	11.58	11.58
7	Rotted manure	.18	.....	8.98	.....	7.53	.....
8	Rotted manure	2.28	1.23	9.33	9.15	10.68	9.10
9	Oat straw	3.83	.....	4.13	.....	11.58	.....
10	Oat straw	.53	2.18	9.78	6.95	14.03	12.80
11	Corn stover	3.62	.....	7.22	.....	3.27	.....
12	Corn stover	2.80	3.21	6.82	7.02	7.22	5.24
13	Timothy hay	3.30	.....	9.30	.....	4.35	.....
14	Timothy hay	4.00	3.85	8.25	8.77	5.05	4.70
15	Cowpea hay	1.05	.....	5.00	.....	6.40	.....
16	Cowpea hay	.....	1.05	4.30	4.65	6.75	6.57
17	Clover hay	3.07	.....	3.02	.....	6.92	.....
18	Clover hay	.57	1.82	5.17	4.09	3.02	4.97

Pot No.	Treatment.	IV.		V.		VI.	
		N. fixed Mg.	Average Mg.	N. fixed Mg.	Average Mg.	N. fixed Mg.	Average Mg.
1	Check	2.80	.....	3.50	.....	7.00	.....
2	Check	4.20	3.50	4.55	4.02	7.70	7.35
3	Horse manure	2.79	.....	2.79	.....	11.24	.....
4	Horse manure	4.24	3.51	4.70	3.74	10.19	10.71
5	Cow manure	7.03	.....	8.08	.....	14.43	.....
6	Cow manure	5.63	6.33	8.78	8.43	10.53	12.48
7	Rotted manure	2.98	.....	4.03	.....	10.03	.....
8	Rotted manure	7.18	5.08	4.73	4.38	13.58	11.80
9	Oat straw	12.63	.....	7.63	.....	6.58	.....
10	Oat straw	4.83	8.73	7.38	7.50	7.66	7.12
11	Corn stover	3.27	.....	5.42	.....	5.77	.....
12	Corn stover	7.17	5.19	6.12	5.79	6.52	6.14
13	Timothy hay	5.75	.....	5.05	.....	8.60	.....
14	Timothy hay	4.70	5.22	9.30	7.17	9.00	8.80
15	Cowpea hay	2.55	.....	6.05	.....	1.80	.....
16	Cowpea hay	5.00	3.77	1.45	3.75	2.85	2.32
17	Clover hay	2.67	.....	3.77	.....	2.72	.....
18	Clover hay	4.47	3.57	3.77	3.77	3.77	3.24

To just what influence of the materials the difference in results was due would be difficult to determine. It may be that the difference in chemical composition of the substances would explain the results. This was the conclusion reached in the ammonification and nitrification experiments and would probably hold true here. It is well known that organic compounds of different composition exert quite different effects on the azotobacter and hence the results from the use of the materials employed

here might be expected, to the extent at least that the different materials had various effects. The important point in this connection which these results bring out is that the character of the compounds present apparently determined the results and the ratio of the nitrogen to carbon present gave no indication of the effects to be expected.

It is interesting to note further that the leguminous hays had much less effect on the azofying power of the soil than the other materials. Especially is this point worthy of mention because of the relative effects of the legumes and non-legumes for green manures. If the latter materials will increase the fixation of nitrogen from the atmosphere by the non-symbiotic azotobacter sufficiently to supply as much nitrogen for the use of crops as is added in legume crops, such materials might frequently be preferable for use on soils. It is impossible from these results to ascertain whether such is the case or not. Further results must be secured with complete field experiments before definite conclusions can be reached.

These results do show, however, that the non-legumes increased the azofying power of the soil to a much greater extent than the legumes. This greater effect was probably due as has been mentioned to the chemical composition of the materials. In this case the effects are in the same direction as the widening of the nitrogen-carbon ratio, and it might seem that the ratio of the materials would indicate the influence on azofication, but inasmuch as the manures of narrower nitrogen-carbon ratio had as much effect as the non-legumes and straws it would evidently not be warranted to draw any conclusion regarding the effects of the ratio in materials added on azofication.

In general then the results show that azofication was favored by manure to a large extent; that straw, stover and non-leguminous hays had almost as great an effect as the manures, although of a much wider nitrogen-carbon ratio, and that the leguminous hays had the least effect of any of the materials used in the experiment. Apparently, the nitrogen-carbon ratio of the materials used was of little or no significance in indicating their influence on azofication and differences in effects were due rather to variations in the chemical compounds present.

There are indications, however, that non-leguminous hays and straws may increase azofication in soils to a large enough extent to make their use more profitable than that of legumes which although adding nitrogen to the soil are somewhat more expensive to use.

These conclusions apply, of course, as must be emphasized again, only to this particular soil type and when the materials are used in amounts such as were employed here, that is, in maximum field applications. The results are, therefore, directly applicable to farm conditions on this soil type and may indicate what will occur on similar soils. Further experiments on the comparative effects of legumes and non-legumes



as green manures from the standpoint of their influence on azofication are extremely desirable and may lead to important practical conclusions.

One point further is worthy of mention in connection with these experiments and that is that the results secured with dextrose were much more satisfactory than those with mannite. The latter material has been considered the best for such work, but it is possible that the cheaper dextrose may serve as well or even better. The point is worthy of consideration in connection with extensive azofication experiments.

Comparing the azofication results as a whole with the ammonification and nitrification results, it appears that there was little similarity in the effects of the different materials on the different processes. Azofication was increased in some cases to a greater extent by some materials than by others, whereas the opposite was the case with ammonification and nitrification.

This fact brings up another important point in connection with the use on soils of organic materials which increase azofication to the greatest extent. Is it necessary that ammonification and nitrification should also be considered? This is a question which must be left for future rather extensive experiments to settle, and involves the whole question of the form in which plants may assimilate their nitrogen, a question which is apparently far from definitely settled as yet.

#### THE CROP YIELDS.

The crop of oats on the pots, the duplicates of which were kept bare for bacteriological tests, was harvested just prior to maturity, dried, ground and analyzed for total nitrogen. The green and dry weights of the crops are given in Table XI and the nitrogen content of the crop together with the calculated removal of nitrogen from the soil are given in Table XII.

TABLE XI.  
THE CROP YIELDS.

Pot No.	Treatment.	Green Weight Gm.	Average Gm.	Dry Weight Gm.	Average Gm.
19	Check .....	189.0	.....	58.6	.....
20	Check .....	207.0	198.00	61.5	60.05
21	Horse manure .....	112.9	112.30	30.3	.....
22	Horse manure .....	119.7	116.30	32.0	31.15
23	Cow manure .....	234.4	.....	70.6	.....
24	Cow manure .....	218.2	226.30	63.0	66.80
25	Rotted manure .....	279.6	.....	85.2	.....
26	Rotted manure .....	298.0	289.00	88.9	87.05
27	Oat straw .....	115.3	.....	32.5	.....
28	Oat straw .....	100.6	107.95	28.9	30.70
29	Corn stover .....	182.9	.....	50.0	.....
30	Corn stover .....	180.7	181.80	52.6	51.30
31	Timothy hay .....	143.2	.....	44.6	.....
32	Timothy hay .....	151.1	147.15	45.3	44.95
33	Cowpea hay .....	260.5	.....	72.4	.....
34	Cowpea hay .....	244.6	252.55	72.8	72.60
35	Clover hay .....	224.9	.....	63.6	.....
36	Clover hay .....	224.9	224.90	64.0	63.80

From examining the yields in Table XI, it appears that the rotted manure, the cow manure and the leguminous hays increased the crop yields to

a considerable extent. The horse manure depressed the yield over that of the untreated soil. The plants in these pots were weak and turned yellow soon after they appeared above the surface of the soil, but after about ten weeks this bad effect from the horse manure disappeared and the oats showed a more vigorous growth. If the experiment had continued longer, it is probable that the yields would have equalled those secured with the other materials. The depressing action was probably due to the introduction with the heavy application of manure of chemical substances which were injurious to the young plants.

All of the non-legume hays, straw and stover materials with a wide nitrogen-carbon ratio gave no increase in the crop yields. In fact, an actual depression in yields occurred. These materials apparently did not decompose sufficiently rapidly to aid the crop grown or the nitrogen content of the soil was more depleted than was believed. At any rate, the legume hays increased the yields, a fact which would indicate that the nitrogen factor on these soils was important and that the non-legumes did not increase the fixation of nitrogen from the atmosphere sufficiently to keep the oats supplied with that element.

The experiment, of course, was continued hardly long enough for definite crop results to be secured and a second crop was planted after the first was removed in order to determine whether different results would be secured, allowing a longer time for the organic material to decompose.

It appears from these first results, however, that the nitrogen-carbon ratio of the organic materials was of considerable significance in determining the effects of the materials used on the crop yields from this particular soil. In every case those substances with the narrower nitrogen-carbon ratios increased to the greatest extent the crop yields, while the materials of wide ratios decreased the crop yields. The nitrogen factor was evidently very important on this particular soil.

TABLE XII.  
THE ANALYSES OF THE CROPS.

Pot No.	Treatment.	N. in Crop %	Average %	C. in Crop %	Average %	N—C Ratio	N. rem'd Gm.	Average Gm.
19	Check .....	.734	.....	39.429	.....	.....	.430	.....
20	Check .....	.730	.732	37.637	38.533	1 : 52.64	.449	.439
21	Horse manure .....	.818	.....	41.911	.....	.....	.248	.....
22	Horse manure .....	.861	.839	37.083	39.497	1 : 47.07	.276	.262
23	Cow manure .....	.734	.....	45.827	.....	.....	.518	.....
24	Cow manure .....	.734	.734	45.372	45.599	1 : 62.11	.162	.490
25	Rotted manure .....	.776	.....	40.413	.....	.....	.661	.....
26	Rotted manure .....	.797	.786	37.367	38.890	1 : 49.48	.709	.685
27	Oat straw .....	.764	.....	38.745	.....	.....	.218	.....
28	Oat straw .....	.771	.767	39.140	38.943	1 : 50.77	.224	.236
29	Corn stover .....	.783	.....	39.195	.....	.....	.392	.....
30	Corn stover .....	.797	.790	37.254	38.225	1 : 48.37	.419	.405
31	Timothy hay .....	.709	.....	38.653	.....	.....	.316	.....
32	Timothy hay .....	.703	.706	39.985	39.319	1 : 55.69	.318	.317
33	Cowpea hay .....	.903	.....	38.372	.....	.....	.654	.....
34	Cowpea hay .....	.868	.885	38.808	38.590	1 : 43.60	.632	.643
35	Clover hay .....	.805	.....	42.410	.....	.....	.512	.....
36	Clover hay .....	.836	.820	44.443	43.427	1 : 52.96	.534	.523

In Table XII, it is seen that the percentage of nitrogen in the oats varied considerably, the tendency being for the lowest yields to show the highest nitrogen content. The largest crops, however, removed the greatest amount of nitrogen from the soil.

The crop yields as a whole show that materials such as were used in this work may exert a considerable influence on bacterial activities and not show the same effect on the crop grown. The effects on subsequent crops, however, would be a more definite indication of the relative values of these materials, because of the need of time for decomposition. In other words, it would not be expected that the effects of such materials on crops would be exerted as soon as effects on bacterial activities. The latter must always precede the former. Hence some time should elapse after applying organic materials before the effect on the crop grown is determined. If the effects of materials of wide nitrogen-carbon ratio are dependent to any extent on the increase in nitrogen content of the soil through non-symbiotic nitrogen-fixation, time should be allowed for this process to occur before the comparative effects on crop yields are tested. It is not regarded, therefore, that these crop yields present facts which oppose in any way the possibility of sufficient azofication occurring in soils treated with non-legumes to equal the effects caused by legumes.

#### THE SECOND CROP YIELDS.

The second crop of oats grown on the same soils as in the case of the first crop was harvested before it had attained any considerable growth. The yields given in Table XIII, however, show some interesting relations to those of the first crop.

TABLE XIII.  
THE SECOND CROP YIELDS.

Pot No.	Treatment.	Green Weight Gm.	Average Gm.	Dry Weight Gm.	Average Gm.
19	Check .....	26.7	.....	6.5	.....
20	Check .....	32.3	29.50	7.7	7.1
21	Horse manure .....	47.7	.....	11.6	.....
22	Horse manure .....	57.0	52.35	12.7	12.15
23	Cow manure .....	49.9	.....	12.8	.....
24	Cow manure .....	57.95	53.92	15.0	13.9
25	Rotted manure .....	34.0	.....	7.4	.....
26	Rotted manure .....	39.5	36.75	9.2	8.3
27	Oat straw .....	41.7	.....	10.0	.....
28	Oat straw .....	49.55	45.62	12.2	11.1
29	Corn stover .....	56.65	.....	11.5	.....
30	Corn stover .....	43.2	49.92	10.0	10.75
31	Timothy hay .....	38.6	.....	7.0	.....
32	Timothy hay .....	43.4	41.00	11.2	9.1
33	Cowpea hay .....	37.3	.....	9.0	.....
34	Cowpea hay .....	51.1	44.20	12.2	10.6
35	Clover hay .....	44.5	.....	9.5	.....
36	Clover hay .....	41.15	42.82	9.5	9.5

In this case, all the treatments increased the oats growth, but the horse and cow manures gave the largest effect here, while the rotted manure gave a smaller effect than any of the other materials. With the first crop, the rotted manure gave the greatest influence, while the cow manure

hardly increased the yield and the horse manure depressed the oats growth. Evidently the cause of the injurious action of the horse manure had disappeared before the second crop was grown, and only beneficial effects from the material were in evidence on the second crop.

The rotted manure had apparently lost much of its value for increasing the crop yield by the time the first crop was removed, and had little effect on the second crop.

The oat straw and corn stover gave greater yields than the legume hays and the timothy hay had only a slightly smaller effect than the clover and cowpeas. It is apparent, therefore, that the conclusion drawn in connection with the first yields was well warranted. The non-legumes here seemed to have a greater or as great an effect on the crop as the legumes. Evidently the nitrogen fixed by azofiers was sufficient to supply the second crop of oats with as much of that element as was furnished by the legumes. Of course, there was probably some neutralizing action here as might be expected. If the first crop of oats took out much more nitrogen where the legumes were used than where the other materials were applied, the second crop might be not as well supplied as in the case of the non-legumes because of a shortage of nitrogen. Such could hardly be the case here, however, to more than a negligible extent, hence the conclusion seems justified that non-legumes may be as beneficial as legumes on crops grown, provided sufficient time is allowed to elapse between the application of the materials and the growth of the crop, for decomposition to occur and the fixation of nitrogen from the atmosphere to take place.

There is much closer agreement between the effects of the various materials on bacterial activities and on the second crop of oats than with the first crop. It will be recalled that the first crop of oats was seeded as soon as the substances were added, and it appears from these results that the influence of many of these common humus-forming substances on crops is much greater if time is allowed for decomposition and for other affected bacterial processes to occur before the crop is grown.

The nitrogen-carbon ratio of the various substances did not seem to be of as much importance in determining their effect on the second crop of oats as with the first crop, although there were indications that the materials with wider ratios had more effect than those with narrower ratios.

#### SUMMARY.

The results of these experiments on this particular soil type lead to the following conclusions.

1. Applications of the common humus-forming materials in maximum amounts for farm conditions and in a dried condition increased bacterial activities, ammonification, nitrification and azofication to a considerable extent.

2. The manures, horse manure, cow manure and rotted manure gave the greatest effect on ammonification in most cases, although timothy hay surpassed the horse manure and cow manure in the extent of its effect in several instances. The oat straw and corn stover had a lesser effect than the manures and the legume hays, clover and cowpeas showed the least effect on ammonification of any of the materials used.

3. Increases in ammonification due to the applications of humus-forming materials were independent of the nitrogen-carbon ratio of the materials added and were probably dependent on the chemical composition of the substances.

4. The relative effects of the various materials used would undoubtedly be somewhat altered for field conditions, because of the fact that they were applied in a dried condition. Especially in the case of the manures would the influence on ammonification be accentuated because of the actual addition of bacteria to the soil.

5. The dried-blood-fresh-soil method gave better results for ammonification than the casein-fresh-soil method. The latter gave better duplicate results, but the differences between different soils were not nearly so pronounced. Some further modification of the casein method seems necessary for its general use.

6. Nitrification was increased in much the same way as ammonification, by the various organic materials. The leguminous green manures exerted, however, somewhat greater effects than the manures, and also more influence than the non-legumes. These results were the opposite of those secured with ammonification, but the differences were not great enough to permit of definite conclusions.

7. Increases in nitrification brought about by the various materials were apparently independent of the nitrogen-carbon ratio in the substances. Indications of a greater effect of materials of a narrower ratio over those of a wide ratio cannot be considered conclusive.

8. Azofication or non-symbiotic nitrogen fixation was favored by manure to a large extent. Straw, stover and non-leguminous hays had almost as great an effect as the manures and the leguminous hays had the least effect of any of the materials used.

9. The nitrogen-carbon ratio of the materials employed were of little or no significance in indicating their effects on azofication. There were indications, however, that non-legumes and straws might increase azofication in soils to a large enough extent to make their use more profitable than that of legumes which add nitrogen to the soil, but are somewhat more expensive to use.

10. Further experiments carried on under field conditions to ascertain the relative effects of legumes and non-legumes on azofication are extremely desirable and results secured may be of great practical importance.

11. Dextrose gave better results in the azofication experiments than mannite and may, therefore, be substituted for the more expensive material.

12. There was little similarity between the effects of the different organic materials on the different bacterial processes. Is it necessary that the material which increases ammonification, nitrification and azofication be chosen for use in soils, or shall an increase in azofying power be sufficient to recommend the substance? This question cannot yet be answered.

13. The manures and legumes increased the first crop of oats, except in the case of the horse manure, which apparently exerted an injurious effect on the crop in its early stages of growth. This injury was disappearing when the oats were harvested and might have been unnoticed had the crop been grown for a longer period.

14. The substances with wide nitrogen-carbon ratio decreased the crop yield while those of narrow ratios gave increases. The nitrogen factor was evidently very important on this soil.

15. The nitrogen-carbon ratio of the organic materials seemed to be of importance in determining the influence on the first crop of oats.

16. If opportunity is to be given for non-legumes to exert as good an effect as legumes, by increasing azofication to a sufficient extent to offset the nitrogen supplied by the legumes, the organic materials must be allowed sufficient time for considerable decomposition to occur before a crop is grown to test the effects.

17. The influence of the various substances applied to the soils was noted on a second crop of oats, but the relative effects were different. The non-legumes had as great an influence as the legumes and hence previous conclusions are confirmed that with the use of the former materials sufficient time must be allowed to elapse for azofication to occur if as beneficial effects are to be secured as with legumes.

18. The nitrogen-carbon ratio of the materials applied to the soil did not seem to be of as much importance in determining the effect on the second crop of oats as in the case of the first crop.

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# CARBON AND NITROGEN CHANGES IN THE SOIL VARIOUSLY TREATED: SOIL TREATED WITH LIME, AMMONIUM SULFATE AND SODIUM NI- TRATE.\*

BY R. S. POTTER AND R. S. SNYDER.

The importance of the organic matter in soils is universally recognized, but the rapidity with which this decomposes and is lost is hardly appreciated. It was recently pointed out by Swanson<sup>19</sup> that 150 tons of vegetation were necessary to produce the organic matter in the surface of some typical Kansas soils. He also estimated that the least amount of organic matter which must be returned to these soils each year in addition to the stubble and corn stalks, in order to maintain their present content of organic matter, was one ton.

Therefore, studies on the rate of decomposition of the organic matter are of interest. This among other things is the object of investigations being carried on by us, and the following paper is the first of a series of reports on experiments with soil treated with various organic and inorganic fertilizers. In general the same points have been investigated, namely: loss of nitrogen as ammonia, evolution of carbon dioxide, and changes in the ammonia, nitrate, nitrogen and carbonate content. This report will be confined to the treatments indicated in the sub-title. The experimental work in connection with the work on stable and green manure is well under way, and will be reported when completed.

Before the details of the experiment are taken up, the investigations of others, pertinent to this work, will be discussed briefly.

## HISTORICAL.

### *Loss of Nitrogen.*

There are on record but few reports of carefully controlled pot experiments showing the effect of lime on the loss or gain of nitrogen. Numerous field experiments have been carried out, and while in general, for practical purposes, field experiments give the more valuable data, yet it is more difficult to control the various factors and hence any effect noted is not certainly due to any one variable.

In 1889 Schloesing<sup>17</sup> introduced into flasks, eight different soils which had previously been growing legumes. The atmosphere was renewed weekly, and at the end of eleven months almost all of the soils showed a slight loss of nitrogen. In no case was there a gain of more than 0.01 gm. nitrogen per kilogram of soil.

In 1891 Berthelot<sup>2</sup> found that clay soils when kept moist gradually lost nitrogen. He gives very few details in connection with his work.

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\*From the Laboratory of Soil Chemistry of the Iowa State Experiment Station.

The most important results in connection with the loss of nitrogen due to the liming of the soil were obtained by Lemmermann<sup>10</sup> and his collaborators. The general plan of their experiments was as follows: Lime was added to the soils at the rate of 0.6 per cent, 1.0 per cent and 1.2 per cent. This corresponds to 6, 10 and 12 tons per acre.\* Ammonium sulfate at the rate of 10 and 20 mg. per 100 gm. soil was used. Their general results and conclusions were as follows:

There was a very slight loss of nitrogen when soils were treated with lime, the greater losses being with the higher amounts. With the heavier treatment of ammonium sulfate and lime there was a considerable loss of nitrogen, while with the smaller treatments, a very slight loss occurred. For practical purposes, then, the results of these investigations show that soils containing, or treated with large amounts of calcium carbonate, will lose part of their nitrogen; but, as the authors point out, such treatments as they have used will but seldom be used in practice. The method by which a loss of nitrogen was detected was simply the determination of the total nitrogen of the soil before and after incubation. Because of the slight differences necessarily dealt with, extremely accurate work was necessary. The above investigators used in all cases the average of eight nitrogen determinations. It is shown in the experimental part of this paper that soil treated with three tons of lime and one-half ton of ammonium sulfate per acre lost an appreciable quantity of ammonia nitrogen, yet not enough to be detected by total nitrogen determinations.

#### *Volatilization of Ammonia from the Soil.*

Berthelot<sup>1</sup> placed moist soil in pots under bell jars in such a manner that the water which condensed on the bell jar ran into receiving vessels. The liquid thus collected was analyzed for ammonia by distillation with magnesia, and the residue was analyzed for total nitrogen. Plants were grown in some of the pots. The soil alone, gave off a very small amount of ammonia and other nitrogen compounds. Still less nitrogen in the form of ammonia and other compounds was collected from the jars with the plants.

Takeuchi<sup>20</sup> carried out an interesting and significant experiment relative to the loss of ammonia when ammonium sulfate is in contact with lime. Pure ammonium sulfate and lime were mixed with varying amounts of water. A current of air was passed through the mixture and then into standard acid. At room temperature but a trace of ammonia was given off.

Hall and Miller<sup>6</sup> found that soils absorb a very small amount of ammonia from the atmosphere. Also more ammonia was collected in vessels containing acid when placed over fields recently manured with am-

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\*Throughout this report, by acre is meant 2,000,000 pounds of soil.



monium sulfate and chloride than when placed over untreated plots. They state that in all their tests it was found impossible to keep the dust from accumulating in the acid vessels, so it appears possible that the increase of ammonia might have been due to the higher content of ammonia in the dust from the ammonia treated plots.

Ehrenberg<sup>4</sup> found that when large quantities of ammonium sulfate and calcium oxide were applied to soils, there was a considerable volatilization of ammonia, which seemed to be influenced by the wind. It was found in the same year that lime might even increase the power of the soil to absorb ammonia.<sup>11</sup> In the following year it was found in other experiments<sup>12</sup> that lime increased the rate of evaporation of ammonia from some soils, and decreased it with others. The procedure by which these results were obtained was to mix the soil with the lime and then pass air through the mixture.

It is thus seen that the data in regard to the action of lime relative to the evaporation of ammonia from the soil are very conflicting. None of the work thus far done has shown absolutely whether or not ammonia, as such, volatilizes from the soil alone and under the application of normal amounts of lime.

#### *Ammonia and Nitrate Transformation.*

The voluminous literature in connection with this phase of the subject will not be gone into here as this investigation was not planned primarily to add anything new to the existing data on the subject. Such data as were taken have been mainly to correlate with the other results.

#### *Evolution of Carbon Dioxide.*

The importance of carbon dioxide in agriculture and its value as a plant food solvent, direct fertilizer, etc., has long been recognized. Many laboratory and field tests of the amount of carbon dioxide produced by the soil and present in the soil atmosphere have been made. Only those which have a direct bearing on the work in hand will be discussed. Formerly the origin of the gas in the soil was thought to be the simple chemical decomposition of the organic matter in the soil, but Wollny<sup>22</sup> has shown by experiments with sterilized soil, that practically all is due directly to the action of micro-organisms and the roots of higher plants.

Stoklasa<sup>18</sup> who did some of the first fundamental work on carbon dioxide production in soil, calculated that certain bacteria produce from two to two and one-half grams carbon dioxide per 100 gm. dry bacteria in one hour. Van Suchtelen<sup>21</sup> who gives an excellent and complete review of the work on carbon dioxide of the soil, measured the amount of the gas given off by various soils under different conditions and compared his results to the numbers of bacteria in the soil. A close relationship was found. His method was as follows: One kilogram of sand was

placed in an eight-litre flask and then 6 kg. of the soil on top of the sand. The carbon dioxide produced was measured by drawing air through the soil by means of a glass tube which reached through the soil down into the layer of sand. He found that the amount of carbon dioxide given varied somewhat with the amount of air passed through the soil, but there was less variation for the larger amounts than for the smaller amounts of air. It was found that much smaller amounts of carbon dioxide were given by soils completely saturated with water than soils somewhat below the saturation point. One-tenth of a gram of ammonium sulfate in 100 gm. soil caused a 500 per cent increase in the carbon dioxide produced. Many other interesting and significant experiments were carried out, and such as are pertinent to our work (to be reported on later) will be discussed at the proper time.

In 1911 Lemmermann<sup>9</sup> and associates published the report of an extensive investigation on the action of calcium oxide and carbonate on the production of carbon dioxide in soils. The plan of their first set of experiments was as follows: One kilogram of the dry soil, after the admixture of the materials to be added, was placed in a flask of about twice the volume of the soil. After making up to 12 per cent moisture, 10 liters of air were passed through the soil for a short time daily and the carbon dioxide collected and weighed. In this set, various organic fertilizers were used in combination with the lime. Curiously, there were no soils untreated with the manures. Since this experiment has more to do with our later work it will only be stated here that in general the limed pots gave off more carbon dioxide than the unlimed after deducting for that bound by the calcium oxide. It was assumed in the case of the soils treated with the carbonate that none of the evolved carbon dioxide came from this carbonate directly. This certainly would not be the case if the soil were at all acid, and it is doubtful if this would be true in any case. Certainly it is a point which should be investigated. In the next series of experiments by these investigators, much the same treatments were given except that calcium oxide alone was used. Instead of measuring the evolved carbon dioxide, the soils were analyzed before and after treatment for carbon by the combustion method. Where no lime or manure was added to the soil, in one case there was a loss of carbon and in the other, a gain. The lime caused a greater loss in both the unmanured and the manured soils than in the corresponding unlimed soils. In the next series calcium carbonate was used. Here again a greater loss of carbon from the limed soil was observed, and also no account was taken of the carbon from the carbonate. In these carbon balance experiments 0.1 per cent and 1 per cent lime were used.

Quite recently there has appeared a report from the Wisconsin Station<sup>6</sup> on the effect of various inorganic fertilizers upon the carbon diox-

ide production in a soil. The only fertilizer used in this instance, which has a direct connection with this work, was ammonium sulfate. One kilogram of soil after being mixed with the salt was placed in a two-liter Erlenmeyer suction flask and after being made up to about 18 per cent moisture the soils were incubated at room temperature for twelve days. Air was drawn through the apparatus for ten minutes every twenty-four hours during this period and the carbon dioxide collected and determined. Amounts of ammonium sulfate from 0.1 gm. to 1 gm. per 100 gm. soil were used. All of the treatments increased the carbon dioxide evolution. The increases were as follows: 0.1 gm. 55 per cent; 0.25 gm. 75 per cent; 0.5 gm. 55 per cent; 1.0 gm. 30 per cent Miami silt loam was the soil used. Nothing else is stated concerning its properties.

#### EXPERIMENTAL.

Several hundred pounds of Miami Silt loam were obtained from one of the station orchards, which is situated on the Wisconsin drift area. The soil is light in color, and low in organic matter. It contains 0.1137 per cent nitrogen and 1.35 per cent carbon. It has a lime requirement according to the Veitch method of 600 pounds of calcium carbonate per acre. After partially air drying, the soil was thoroughly mixed and then allowed to air dry completely. All but 80 to 90 pounds was placed in a tin storage can and reserved for the remaining experiments in this series. The smaller sample was spread out, thoroughly mixed again and exactly 1134 gm. (2½ pounds) was weighed out into glass pots whose height was 11.5 cm. and whose diameter was 11.5 cm. At the same time a small sample of the soil was drawn and sealed up in a Mason jar.

The general arrangement of the apparatus is shown in Figure 1 and the detailed arrangement in Plate I. The current of air, drawn by a water pump, first enters the flask A containing concentrated sulfuric acid, which takes the water and ammonia from the air. The object of removing the water is to keep the soda lime tube from clogging. The air then passes through tube B and C. Tube B holds 200 gm. of soda lime and tube C 1.5 kg. The soda lime in B is renewed every two or three days during the course of an experiment. The current of air is then divided, one-half going through D containing sulfuric acid of such a strength that it gives a partial pressure of water vapor about equal to the partial pressure of water vapor in the atmosphere of Iowa during the summer months. The other half of the air current goes through apparatus in all respects like that traversed by the half entering D. The current of air, after leaving D, goes through twelve tubes, each leading to a bell jar. Only one of these is shown in Fig. 2, but they are all arranged in exactly the same manner. The air, entering the bell jar E, which is 35 cm. in height and 15 cm. in diameter, passes over the soil in the pot F, bubbles through standard acid in G, and through 5 per cent potassium hydroxide in

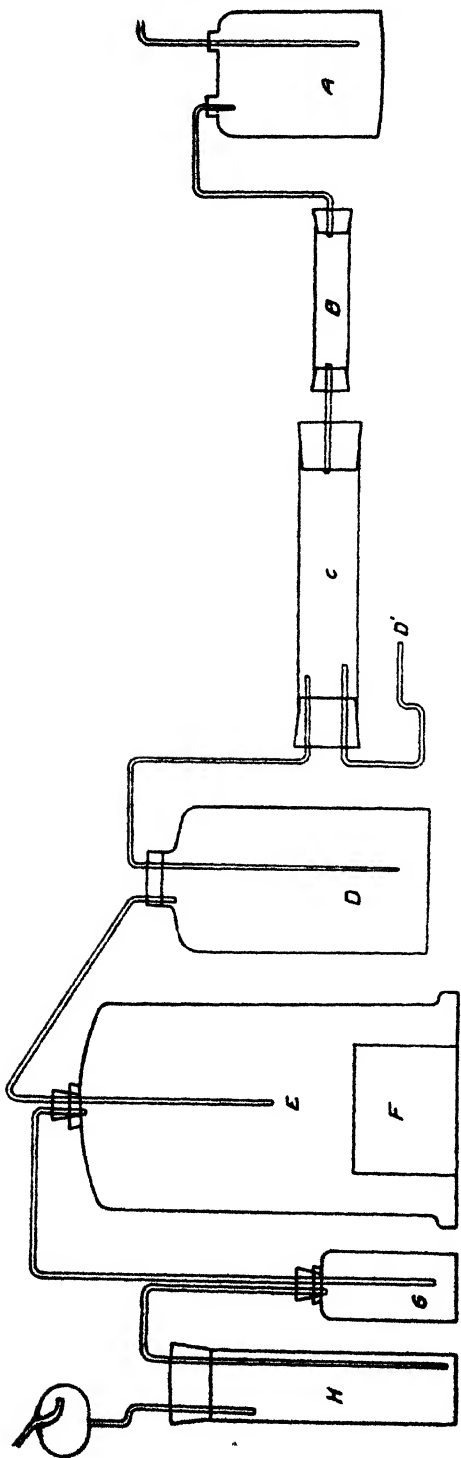


Figure 1—Diagram of Apparatus Used in Determining Carbon and Nitrogen Changes in Soil Variously Treated.

H. H consists of a glass cylinder 20 cm. by 3.5 cm. The rubber stopper closing it bears a 25 cc. Kjeldahl connecting bulb. It is placed at such a height that when the air is passing through it, it is filled with alkali up to within a few mm. of the end of the bent exit tube. This makes a simple and effective carbon dioxide absorption tower. To test its efficiency two of these towers were connected in series to a bottle containing a dilute solution of sodium bicarbonate. A current of air was drawn through the system at about the rate used in all our work and very dilute acid was slowly dropped into the sodium bicarbonate solution. After one day the carbon dioxide was determined in the alkali in each bottle. After deducting the blank for the alkali, the tower next the bicarbonate solution contained 350 mg. carbon dioxide, while the next tower contained 0.9 mg. This was considered a sufficiently complete absorption for our purposes. The treatment of the pots of soils in tons (T) and pounds per acre was as follows:

POT NO.	TREATMENT.
1, 2, 13, 14	Check.
3, 4, 15, 16	3 T. $\text{CaCO}_3$
5, 6, 17, 18	1285 lbs. $\text{NaNO}_3$
7, 8, 19, 20	1000 lbs. $(\text{NH}_4)_2\text{SO}_4$
9, 10, 21, 22	3 T. $\text{CaCO}_3$ ; 1285 lbs. $\text{NaNO}_3$
11, 12, 23, 24	3 T. $\text{CaCO}_3$ ; 1000 lbs. $(\text{NH}_4)_2\text{SO}_4$

Just before starting the experiment air was drawn through the apparatus for an hour to free it of carbon dioxide. While this was being done 3.4 gm. lime (precipitated calcium carbonate) were added to the designated soils and very thoroughly mixed. The soil, whose saturation capacity for water was 34 per cent, was made up to 22 per cent moisture as follows: First a fresh soil emulsion was prepared by mixing 400 gm. of fresh field soil with four liters of ammonia and carbon dioxide free water. After allowing the soil to settle a few minutes three liters were decanted and then 100 c.c. were pipetted into each of twenty-four 250 c.c. flasks, numbered 1 to 24 to correspond to the pot numbers. Solutions of ammonium sulfate and sodium nitrate of such a strength that 100 c.c. contained the required amount (0.5670 gm. and 0.7393 gm. respectively) were prepared. Quantities of 100 c.c. of each of these solutions were pipetted into the proper flasks and then all the flasks were made up to the mark with ammonia and carbon dioxide free water. After being shaken, the mixtures were poured into their respective soils, which were then immediately transferred\* to the proper bell jars. The current of air was

\*In all the later work the procedure at this point was somewhat different. All the dry ingredients were mixed with the soils and the pots containing the soils were placed under the bell jars. Carbon dioxide free air was then passed through the apparatus for an hour or more. Then the 250 c.c. of water (emulsion, etc.) was added to the soil by means of a long funnel. By the original method of procedure, no doubt, there was a slight loss of carbon dioxide, but by comparison with work done by the second method of procedure it was found that this loss was relatively negligible.

turned on and run continuously throughout the period of the experiment. The experiment was started October 31, 1914. The alkali was transferred and the carbon dioxide estimated on the days shown in Table I. Pots Nos. 1 to 12 were run until November 27 and Nos. 13 to 24 until January 18. Only the carbon dioxide results for the pots which were run the longer period are reported. Only two titrations were run on the other set and these agreed very closely with those given.

Besides the determination of the evolved carbon dioxide, the ammonia liberated was determined. The original intention was to estimate the ammonia and carbon dioxide at the same time, but the amount of the former given off was so small this plan was abandoned. In this experiment 0.02 N acid was used as the absorbent. For the soils Nos. 1 to 12 this was titrated directly with 0.02 alkali. Afterwards it was thought that part of the "ammonia" thus found was due to alkali dissolved from the bottle. Therefore for the remaining soils, at the completion of the "run" the acid was transferred to Kjeldahl flasks, made alkaline and the ammonia aerated into standard acid. The results apparently confirmed the suspicion that part of the alkalinity was due to the bottles.

#### METHODS OF ANALYSIS.

##### *Total Nitrogen*

Twenty-five gm. of soil were used for each determination. The salicylic acid method was used, the reduction being carried out by powdered zinc and the digestion after the Kjeldahl-Gunning method. Instead of distillation of the ammonia it was determined by the aeration method of Kober and Graves.<sup>8</sup> Probably because of the insoluble residue in soil digestions we have found it necessary to aerate these solutions longer than the originators of the method advised. Using a current of air of about 500 liters an hour for two and one-half hours was found to recover all of the ammonia, but for certainty all our aerations were run for three and one-half hours or more. Only by extreme care in every operation was the probable error of the determinations reduced as low as it was. By this method, working with this and other soils, we were able to determine all of 150 parts per million of nitrate nitrogen added to the soil. All determinations were made in quadruplicate.

##### *Ammonia Nitrogen.*

This was determined by the method proposed by the authors.<sup>14</sup> It was thought that in the case of limed soils there would be a loss of ammonia upon air drying. Some of the limed samples containing the larger amount of ammonia were analyzed in the moist condition and also after quickly air drying by the aid of an electric fan. In all cases the same amount of ammonia was found before and after the drying. All determinations were made in duplicate.

*Nitrate Nitrogen.*

Nitrates were determined by the modification of the aluminum reduction method as proposed by Burgess<sup>3</sup> using the apparatus proposed by us.<sup>15</sup> The determinations were always made on the wet soil within two hours after removing from the pots.

*Carbonates.*

The determination was made by the method of MacIntire and Willis,<sup>18</sup> using a somewhat different apparatus than that used by them. A Kjeldahl flask contained the soil and the absorption tower was just like the one used in the main part of the experiment. According to our experience the atmospheric blank was not sufficiently constant to be included in the alkali blank. In all cases the apparatus was carefully freed from carbon dioxide. One part of 85 per cent phosphoric acid to fifteen parts of water was used. This was shown by the sponsors of the method to decompose quantitatively calcium carbonate in the soil.

In Table I are found the results of the carbon dioxide determinations, expressed in pounds per acre.

It will be observed that there are no results given for the time between December 11 and 19. During that time the apparatus sprung a leak at such a place that it was not apparent to us, and all the results were high for that period. The degree of closeness with which the duplicate soils agreed is considered satisfactory. Something more in this connection should be said in regard to the method of drawing out the carbon dioxide from the soil. It will be observed from the historical section of this paper that all of the previous work involved drawing the air either over or through the soil during only a short period once a day. In our work we attempted to have the air current the same over each pot, but this was adjusted only by observing the rate of bubbling of the air. This, at best, would give currents of air only approximately the same. The amount of air going over in twenty-four hours was from 25 to 50 liters. It was in these and later experiments observed that if one of the streams of air became partially or completely stopped for perhaps a day and then turned on fast for several hours before estimating the carbon dioxide the amount of the gas given off by this pot would be lower than its duplicate, and the deficit would never be made up. On the other hand, if one of the streams of air was turned on 5 or 10 times as fast as its duplicate, if the latter ran normally the duplicates would check as closely as ordinarily. It was recently<sup>16</sup> found that the content of carbon dioxide in the soil atmosphere was not appreciably changed by high and continuous winds. In view of these facts it appears that experiments involving the drawing out of the carbon dioxide only once or even twice would not give correct results. There would undoubtedly be a lessened production

TABLE 1.  
CARBON DIOXIDE DETERMINATIONS.  
(Expressed in terms of pound per acre.)

Pot No.	Treatment	Oct. 31-Nov. 2	Nov. 2-6	Nov. 6-13	Nov. 13-20	Average	Nov. 20-27	Average	Nov. 27-Dec. 4	Average	Dec. 4-11	Average	Dec. 11-19	Dec. 19-31	Average	Dec. 31-Jan. 8	Jan. 8-18	Average	Total	Average
13	Check.	287	220	258	148	143	144	130	126	113	111	201	(138)c	205	203	(153)a	190	187	1981	1960
14	Check.	274	280.5	226	223	249	149.5	145	126	109	109	205	(138)c	205	203	(153)a	184	187	4165	4136
15	Lime.	1205	440	506	256	221	227	(227)a	227	(175)a	175	(360)a	(231)c	360	360	(247)a	(295)a	295	4107	4136
16	Lime.	1163	439	474	257	236	256.5	112	112	95	94	151	(108)c	151	149	118	148	295	1653	1656
17	NaNO <sub>3</sub>	282	171	214	130	129	127	116	114	93	94	147	(108)c	147	149	123	150	149	1659	1656
18	NaNO <sub>3</sub>	272	180	212	128	126	127	109	109	(103)a	103	161	(121)c	161	170	130	159	159	1730	1715
19	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	308	161	222	130	126	126	(109)a	109	103	103	179	(121)c	179	170	127	156	156	3778	3778
20	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	273	290	214	130	126	126	208	208	194	194	280	(214)c	280	280	196	260	260	3778	3778
21	Lime & NaNO <sub>3</sub>	1109	343	490	241	243	242	202	205	196	195	(380)a	(214)c	280	280	196	260	260	3878	3828
22	Lime & NaNO <sub>3</sub>	1165	1137	478	259	250	241	242	205	196	195	(214)c	(214)c	280	280	196	260	260	3878	3828
23	Lime & (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	1083	347	(480)b	(240)b	210	210	154	154	139	141	248	(168)c	248	243	175	200	200	3444	3444
24	Lime & (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	1079	1081	354	(480)b	218	214	154	154	141	140	238	(168)c	238	243	171	203	203	3446	3445

(a) Value assumed to be the same as duplicate.

(b) Value taken arbitrarily from general direction of curves.

(c) Value taken directly from the curves.



of the gas under these conditions. It is not certain just why this should be so but the increase in the partial pressure of the carbon dioxide acting according to the mass action law would slow up the production and then the excess carbon dioxide would tend to be toxic to the bacteria. The current of air in our experiments assuming thorough mixing in the bell jar maintained an air mixture having a partial pressure of carbon dioxide somewhat below that in the normal atmosphere.

The results for the various periods have been divided by the number of days in the period. This gives the average amount of gas given off per day for the respective period. The results found in Table II have been plotted and are shown in Figure 2. The curves as plotted carry the assumption that the average amount of carbon dioxide given off per day each period was being given off at that rate in the exact middle of the period. While not absolutely justifiable, some such an assumption seems necessary. The points of the curve between December 11 and December 19, which were lost have been assumed to lie on a straight line between the two points before and after this time. This is the best that can be done and is no doubt not far from correct because the general tendency of all the curves is to be in straight lines.

TABLE II.  
AVERAGE AMOUNT OF CARBON DIOXIDE GIVEN OFF PER DAY IN SOIL  
VARIOUSLY TREATED.

Treatment	Pot. No.	Oct. 31-Nov. 2	Nov. 2-6	Nov. 6-13	Nov. 13-20	Nov. 20-27	Nov. 27-Dec. 4	Dec. 4-11	Dec. 19 31	Dec. 31-Jan 8	Jan. 8-18
Check .....	13, 14	140	55.5	36.1	21.3	20.6	18.3	15.9	18.5	19.1	18.7
3 T. $\text{CaCO}_3$ .....	15, 16	593	109.7	69.7	36.6	32.6	32.7	25.0	32.7	30.9	29.5
1285 lbs. $\text{NaNO}_3$ .....	17, 18	138.5	43.7	30.2	18.4	18.1	16.3	13.4	13.5	15.0	14.9
1000 lbs. $(\text{NH}_4)_2\text{SO}_4$ .....	19, 20	245	40.5	31.1	18.6	18.0	15.7	14.7	15.5	16.0	15.7
3 T. $\text{CaCO}_3$ + 1285 lbs. $\text{NaNO}_3$ .....	21, 22	568.5	88.2	69.1	35.7	37.6	29.3	27.9	25.5	26.6	26.4
3 T. $\text{CaCO}_3$ + 1000 lbs. $(\text{NH}_4)_2\text{SO}_4$ .....	23, 24	540.5	87.5	lost	lost	30.6	22.0	20.0	22.1	21.6	20.1

The most striking thing shown by the curves is the rapidity with which they drop to nearly horizontal lines. This is not surprising for the limed soils but the reason is not so apparent for the unlimed. That it is not due to the freeing of carbon dioxide mechanically held in the soil is shown by the analysis of the soil for carbonates. By the analysis of the original soil 318 pounds per acre of carbon dioxide were found. In the case of the check pots if it is assumed that the normal carbon dioxide production is 20 pounds a day after November 13, then the excess during the first thirteen days was 495 pounds. Therefore if all of the carbon dioxide found by the analysis of the soil were mechanically held and all of it were given off in the first few days, neither of which assumptions is

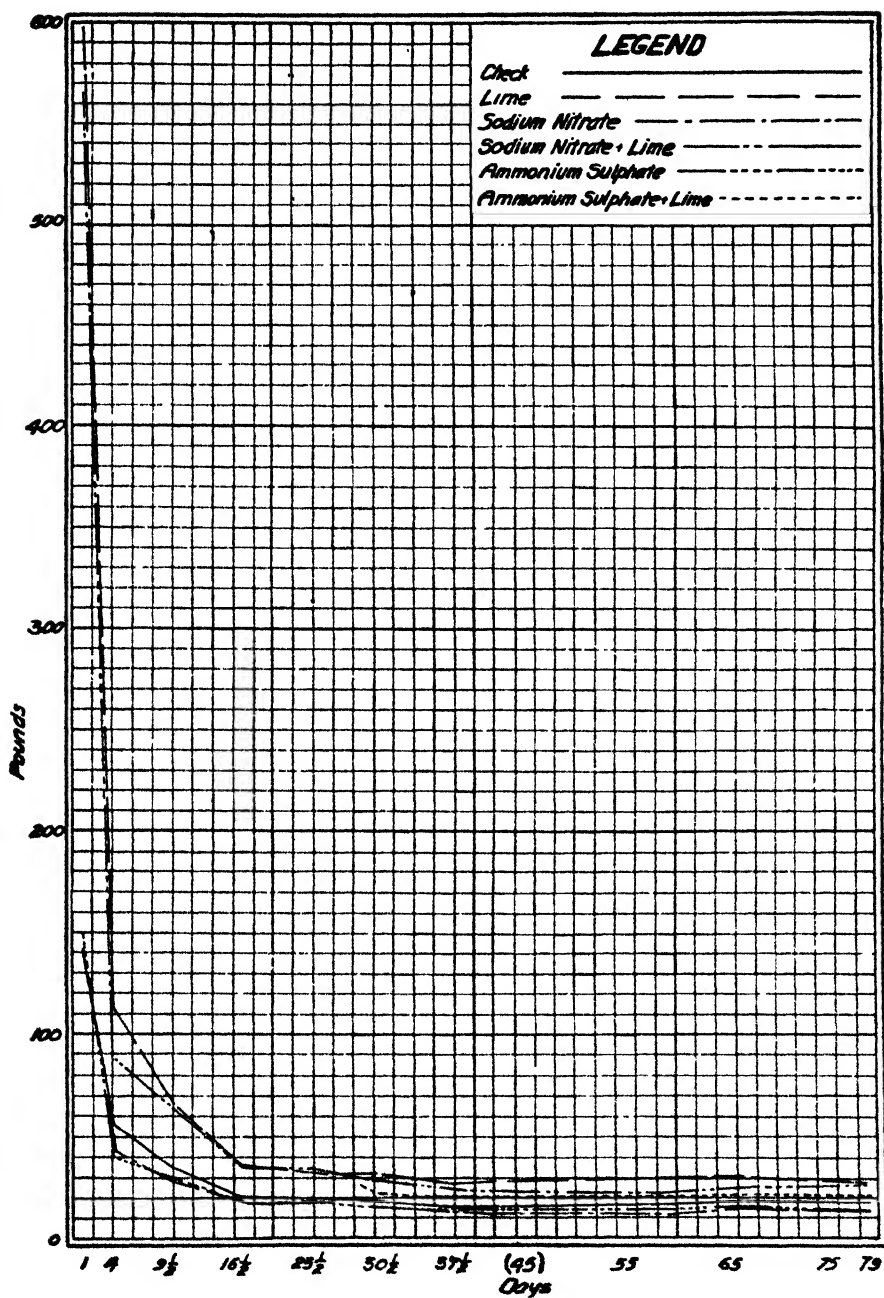


Figure 2—Diagram Showing the Average Amount of Carbon Dioxide given off per Day in Soil Variously Treated.

probable, there would still be an excess of 177 pounds given off during the first thirteen days.

It has been shown by Klein <sup>7</sup> that the carbon dioxide production of soils is enhanced by previous drying and that the water soluble matter is increased in a clay loam soil low in organic matter. Perhaps the increase in the water soluble material, making a richer nutrient solution, is the cause of the increased carbon dioxide production. After a few days this rich nutrient solution would reach its normal concentration for the particular soil and the bacterial activities would be somewhat lessened. It was recently shown by Russell and Appleyard <sup>18</sup> that an increase in the carbon dioxide in the soil atmosphere of field soils after rains was always accompanied by a decrease in nitrates. They, therefore, concluded that the oxygen of the nitrates was utilized in the oxidation processes. In the soil used there were 44.3 pounds of nitrate nitrogen or 217 pounds of sodium nitrate. This might have served to enhance the carbon dioxide production. Still another factor which might have contributed to the initially large amount of carbon dioxide evolved is the fact that previous drying of the soil alters the colloidal condition of the soil, permitting an enhanced rate of oxidation.<sup>7</sup>

The data in regard to the carbonate content of the soils are given in Table III. The values which are given are the average of two closely agreeing duplicates. The results are expressed in pounds per acre of carbon dioxide and calcium carbonate.

TABLE III.  
CARBONATE CONTENT OF SOILS.

Soil	CO <sub>2</sub> in lbs. per A.	Average	CaCO <sub>3</sub> in lbs. per A.	Average	Soil	CO <sub>2</sub> in lbs. per A.	Average	CaCO <sub>3</sub> in lbs. per A.	Average
Original	198	.....	676	.....	13	115	.....	261	.....
1	170	.....	386	.....	14	115	115	261	261
2	170	170	386	386	15	lost	.....	.....	.....
3	548	.....	1245	.....	16	492	492	1118	1118
4	540	544	1225	1235	17	49	.....	1112	.....
5	40	.....	90.8	.....	18	58	53.5	1430	1276
6	36	38	81.6	86.2	19	53	.....	1316	.....
7	36	.....	81.6	.....	20	58	55.5	1430	1373
8	40	38	90.8	86.2	21	492	.....	1118	.....
9	448	.....	1016	.....	22	496	494	1126	1124
10	545	496.5	1238	1127	23	386	.....	876	.....
11	282	.....	640	.....	24	381	383.5	865	870.5
12	339	310.5	770	705					

The carbon dioxide which is the direct result of the decomposition of organic matter was also computed. Where A is the carbon dioxide evolved, B that originally present in the soil, including that added in the lime, and C that left in the soil, and X is that which is the direct result of organic decomposition, then

$$A-(B-C)=X.$$

The data so obtained are given in Table IV.

TABLE IV.  
COMPOSITION OF CARBON DIOXIDE EVOLVED FROM ORGANIC  
DECOMPOSITIONS.

Treatment	Soil	CO <sub>2</sub> added in CaCO <sub>3</sub> lbs. per Acre	CO <sub>2</sub> from Organic Matter lbs. per Acre
Check .....	13, 14	.....	1787
3 T. CaCO <sub>3</sub> .....	15, 16	2,637	1693
1285 lbs. NaNO <sub>3</sub> .....	17, 18	.....	1411.5
1000 lbs. (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	19, 20	.....	1472.5
3 T. CaCO <sub>3</sub> + 1285 lbs. NaNO <sub>3</sub> .....	21, 22	2,637	1387
3 T. CaCO <sub>3</sub> + 1000 lbs. (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	23, 24	2,637	893.5

The results as given in Table IV are, to say the least, astonishing. Lime has time and again been proven to increase the bacterial activity of soils, yet in this case there is less decomposition in the case of the limed pots than in the unlimed. Perhaps if the experiment had been run for a longer time the results would have been different. This would be true if the curves for the carbon dioxide production had continued in a horizontal direction as the last few points indicated would be the case. In fact, in another experiment to be reported on later it was found in the case of four pots made up with the same soil and exactly like pots Nos. 1 to 4 in this series, except that they were run 18 weeks, that the limed soils did give off more carbon dioxide from the organic material than did the unlimed. Just why the increase does not manifest itself immediately is not certain. It is possible that the large amount of the gas given off by the limed pots inhibits the action of the bacteria until it has diffused from the soil. It is believed that the experimental error involved in the determination of the evolved carbon dioxide is quite small, but the error in the determination of the residual carbon dioxide in the soil is relatively large. An error in the titration of 0.1 c.c. for instance, makes an error of 17.7 pounds of carbon dioxide per acre. But the greatest variation of any of the duplicate titrations of the carbon dioxide in these soils was just 0.1 c.c., so it seems that the variations in the above table are well outside the experimental error. However, the values for the pots 23 and 24 involving so many assumed values might quite possibly be too low. Our results for ammonium sulfate are certainly contrary to those of Van Suchtelen<sup>21</sup> and Fred and Hart.<sup>5</sup> The soil used by the two later investigators was a Miami Silt Loam, the same type as ours, yet there might have been decided differences in organic matter content, reaction, etc. The great differences are, no doubt due to the differences in the soil flora. It is also observed that the sodium nitrate treated pots show a decreased carbon dioxide production. The reason for this is not clear. The only

apparent explanation is that sodium nitrate at this concentration is toxic. This however is hardly probable.

In Table V the ammonia evolved by the soils will be considered. The results are expressed in pounds of nitrogen per acre. Except for pots 23 and 24 all the duplicate soils checked quite closely, hence for them only the averages are given.

TABLE V.  
AMMONIA EVOLVED FROM SOILS.

Treatment	Soil	Ammonia Nitrogen lbs. per Acre	Soil	Ammonia Nitrogen lbs. per Acre
Check .....	1, 2	0.5	13, 14	0.35
3 T. $\text{CaCO}_3$ .....	3, 4	0.55	15, 16	0.25
1285 lbs. $\text{NaNO}_3$ .....	5, 6	0.5	17, 18	0.30
1000 lbs. $(\text{NH}_4)_2\text{SO}_4$ .....	7, 8	0.85	19, 20	0.30
3 T. $\text{CaCO}_3$ + 1285 lbs. $\text{NaNO}_3$ .....	9, 10	0.85	21, 22	0.35
3 T. $\text{CaCO}_3$ + 1000 lbs. $(\text{NH}_4)_2\text{SO}_4$ .....	11, 12	1.5	23	2.4
			24	2.95

The losses while small were definite. For instance, one c.c. of 0.02 N. ammonia is equivalent to 0.5 pound of nitrogen per acre. As pointed out in the introduction, no doubt Nos. 1 to 12 are too high. The small amount of ammonia nitrogen lost from the limed pots is interesting. The loss of 2.7 pounds from pots 23 and 24 in the 12 weeks, if kept up would mean a loss of about 11 pounds during the year. However, as shown by Table VI the ammonium sulfate is gradually becoming transformed, so that the loss after the 12 weeks would be no greater than the soils receiving no ammonium sulfate.

In Table VI the amounts of ammoniacal and nitrate nitrogen are given. They are expressed in pounds of nitrogen per acre.

It will be recalled that 212 pounds of nitrogen was added to pots 5 to 12 and 17 to 24. The above data are of interest because, to the knowledge of the authors, they are obtained from the only experiment of its kind, showing the transformation of ammonium sulfate and sodium nitrate using an absolute method for ammonia. In all other like experiments the magnesia method or the hydrochloric acid extraction method have been used. The former gives too high results and the latter too low.

The results from the above table suggest a possible reason for the depression of the carbon dioxide production in the ammonium sulfate treated pots. In the case of these soils it seems that in no instance has it all been nitrified, which shows that at least ammonification has not been increased by the presence of the nitrate. Therefore it is possible that there has been such an increase in the nitrification that the number of the bacteria has been reduced and hence carbon dioxide production inhibited. It is a well known fact that the nitrifiers can live and multiply in a medium free from organic matter. If, as our results show, there is a depression

in the carbon dioxide production, due to the application of ammonium sulfate, this need cause no deleterious result for the nitrifiers are apparently considerably activated. The finding of less nitrate nitrogen in the sodium nitrate treated pots than actually had been added is not, of course, necessarily due to denitrification in the narrow sense of the term; it is more probably due to assimilation.

TABLE VI.  
NITROGEN IN THE SOILS AS AMMONIA AND NITRATE.

Treatment	Soil	Ammonia as lbs. Nitrogen per A.	Nitrate as lbs. Nitrogen per A.	Average	Excess over the corres- ponding Unfertilized Pots
	Original	12.6	44.3	.....	.....
Check .....	1	11.4	60.7	.....	.....
(ditto) .....	2	18.8	60.7	60.7	.....
3 T. $\text{CaCO}_3$ .....	3	14.8	68.8	.....	.....
(ditto) .....	4	14.8	72.1	70.4	.....
1285 lbs. $\text{NaNO}_3$ .....	5	11.4	219.8	.....	.....
(ditto) .....	6	12.4	218.1	218.9	158.2
1000 lbs. $(\text{NH}_4)_2\text{SO}_4$ .....	7	13.6	104.8	.....	.....
(ditto) .....	8	13.4	104.8	104.8	44.1
3 T. $\text{CaCO}_3$ + 1285 lbs. $\text{NaNO}_3$ .....	9	17.2	209.8	.....	.....
(ditto) .....	10	14.6	209.8	209.8	139.4
3 T. $\text{CaCO}_3$ + 1000 lbs. $(\text{NH}_4)_2\text{SO}_4$ .....	11	11.6	196.8	.....	.....
(ditto) .....	12	16.4	196.8	196.8	126.4
Check .....	13	13.4	82.4	.....	.....
(ditto) .....	14	13.2	83.4	82.9	.....
3 T. $\text{CaCO}_3$ .....	15	lost	lost	.....	.....
(ditto) .....	16	13.0	113.4	113.4	.....
1285 lbs. $\text{NaNO}_3$ .....	17	18.1	275.2	.....	.....
(ditto) .....	18	19.3	283.	279.1	196.2
1000 lbs. $(\text{NH}_4)_2\text{SO}_4$ .....	19	34.0	257.6	.....	.....
(ditto) .....	20	36.2	261.2	259.4	176.5
3 T. $\text{CaCO}_3$ + 1285 lbs. $\text{NaNO}_3$ .....	21	14.6	323.6	.....	.....
(ditto) .....	22	14.6	322.4	323.0	209.6
3 T. $\text{CaCO}_3$ + 1000 lbs. $(\text{NH}_4)_2\text{SO}_4$ .....	23	18.1	305.6	.....	.....
(ditto) .....	24	18.6	306.8	306.2	192.8

As mentioned in the introduction, all total nitrogen determinations were carried out in quadruplicate. In the second column of Table VII the average and the probable error of these determinations are given. In the third column the average of the duplicate pots are given. In the next column are the corrected values. There is a slight correction to be applied to the limed soils. Assuming that after the experiment 2 gm. of lime remained, due to the "dilution" of the soil by this amount, 0.0002 has been added to each value. The amount of nitrogen added to the respective pots, 0.01060 per cent has been subtracted. While the gain or loss given in the last column, in most cases is small, yet it is thought dependence can be placed in the ones which are greater than 0.0010. The untreated soils in the case of the four weeks' run show a slight loss and no change for the twelve weeks. The limed pots in both cases show a slight gain. The unlimed sodium nitrate treated pots both show a loss, and the four and twelve weeks' results being so nearly alike indicate that the loss all came at first. The total nitrogen of the limed sodium nitrate

pots is unchanged, indicating that the lime has done away with the loss of nitrogen, which loss might or might not have been due to denitrification.

There are only slight changes in the ammonium sulfate treated pots. The loss of ammonia from soils 23 and 24, computed to the same basis as this table was 0.00013. It is seen, therefore, that to check the loss of ammonia from soil by total nitrogen determination is impossible unless much larger losses are involved than was found in this work.

TABLE VII.  
DETERMINATION OF NITROGEN CONTENT OF SOILS.

Treatment	Pot No.	Nitrogen in per cent. of Oven Dry Soil	Average	Average Corrected	Loss or Gain
Check	1	.11228 ± .00028			
(ditto)	2	.11277 ± .00032	.11253	.1125	— .0012
3 T. CaCO <sub>3</sub>	3	.11484 ± .00011			
(ditto)	4	.11488 ± .00019	.11482	.1150	+ .0013
1285 lbs. NaNO <sub>3</sub>	5	.12129 ± .00010			
(ditto)	6	.12176 ± .00013	.12153	.1109	— .0028
1000 lbs. (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	7	.12630 ± .00021			
(ditto)	8	.12632 ± .00022	.12631	.1157	+ .0020
3 T. CaCO <sub>3</sub> + 1285 lbs. NaNO <sub>3</sub>	9	.12384 ± .00016			
(ditto)	10	.12575 ± .00005	.12479	.1144	+ .0007
3 T. CaCO <sub>3</sub> + 1000 lbs. (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	11	.12370 ± .00039			
(ditto)	12	.12307 ± .00016	.12338	.1130	— .0007
Check	13	.11370 ± .00000			
(ditto)	14	.11379 ± .00021	.11374	.1137	.0000
3 T. CaCO <sub>3</sub>	15	lost			
(ditto)	16	.11428 ± .00016	.11428	.1145	+ .0008
1285 lbs. NaNO <sub>3</sub>	17	.12202 ± .00021			
(ditto)	18	.12150 ± .00059	.12176	.1112	— .0025
1000 lbs. (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	19	.12412 ± .00000			
(ditto)	20	.12598 ± .00010	.12505	.11445	+ .00075
3 T. CaCO <sub>3</sub> + 1285 lbs. NaNO <sub>3</sub>	21	.12381 ± .00010			
(ditto)	22	.12465 ± .00018	.12423	.1138	+ .0001
3 T. CaCO <sub>3</sub> + 1000 lbs. (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	23	.12368 ± .00010			
(ditto)	24	.12340 ± .00000	.12354	.1131	— .0006
Original		.11370 ± .00022		.1137	

#### SUMMARY AND CONCLUSIONS.

1. In conclusion it should be stated that we are at this time planning some rather exhaustive investigations into the relation of the amount of carbon dioxide given off by soils to the manner and speed of drawing the air over and through the soil. As stated earlier in this paper, it seems that some such method as we have used would give results more typical of field conditions than where the air was drawn *through* the soil. Possibly the rate at which the air is drawn over the soil is of considerable importance. These and other points are being investigated. Until these points are settled too great emphasis will not be placed on the relative amounts of carbon dioxide evolved in this experiment.

2. For all the soils except those treated with both ammonium sulfate and lime, about 0.3 pound of ammonia nitrogen was given off in the

twelve weeks. If kept up throughout the year this would mean a loss of a little over a pound per acre in a year, an insignificant amount when compared to that lost by leaching, cropping, etc. The loss from the soils treated with both lime and ammonium sulfate was about ten times as high for the period of the experiment, but it is not at all probable that this rate would be held for a very long period after the application of the sulfate. Therefore, we can say with considerable assurance that the danger of loss of ammonical nitrogen from the soil of the type used here is practically negligible.

3. In a general way, the total nitrogen determinations show there is a smaller loss or a greater gain of nitrogen for the limed soils than the corresponding unlimed soils. Whether the losses are due to denitrification or not, it cannot be said. The loss of nitrogen from the sodium nitrate treated soils and the gain of nitrogen in those soils treated with both sodium nitrate and lime, points to denitrification, yet this seems improbable, for the soil in question is a soil low in organic matter, in good physical condition and not water logged. But in whatever manner the nitrogen was lost there are points which must be investigated before results obtained as have these can be applied to field conditions.

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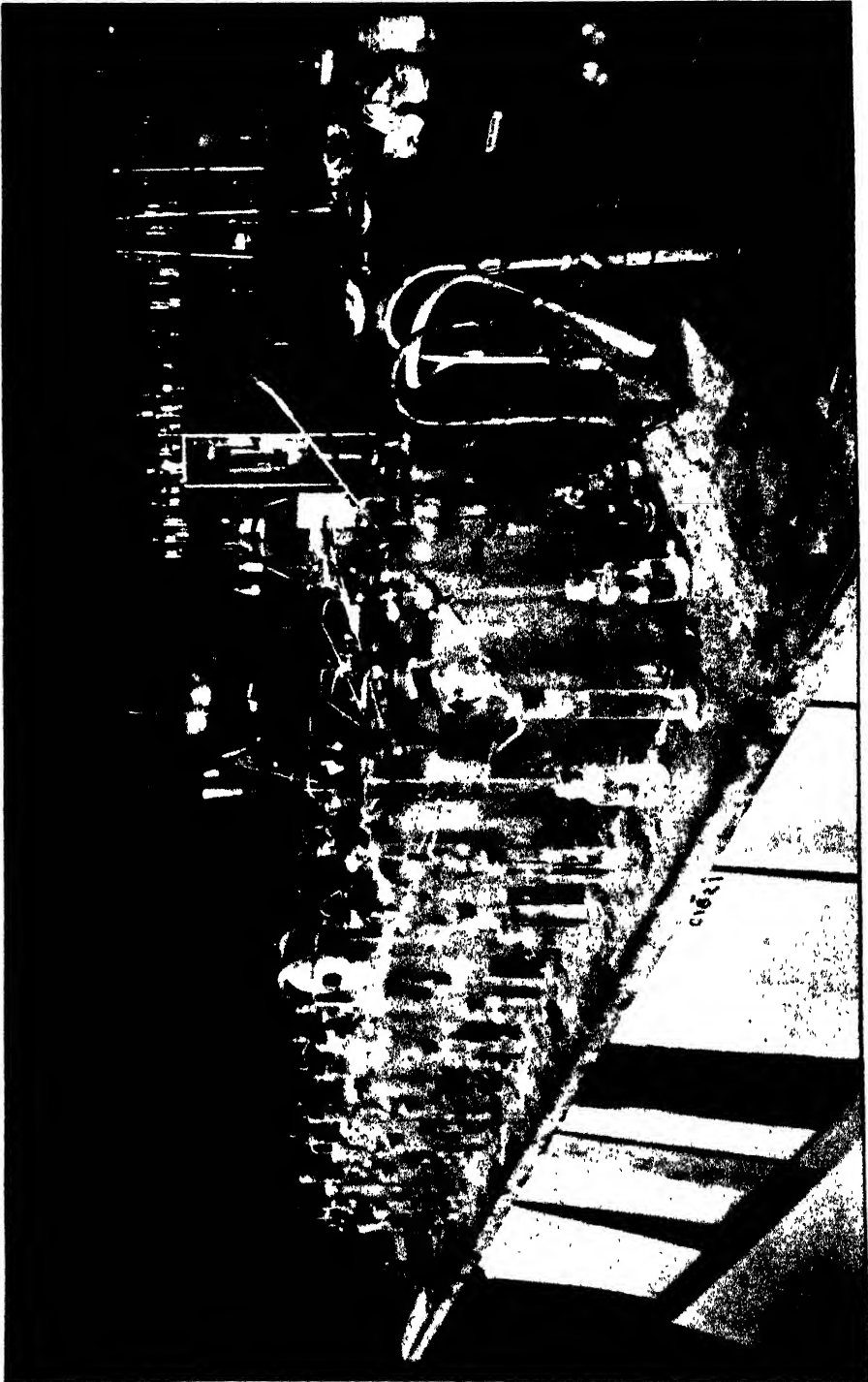


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**PLATE I**

**Apparatus Used in Determining Carbon and Nitrogen Changes in Soil Variously  
Treated.**





# EFFECT OF GRINDING ON THE LIME REQUIREMENT OF SOILS.\*

By R. C. Cook.

In applying the Veitch method for the determination of soil acidity, a question often arises as to the fineness of the soil sample. Obviously, the use of a fine sieve will make necessary the elimination of a large part of the soil and hence give misleading results when calculated to the acre basis. On the other hand, the use of a sieve with meshes too large may be equally objectionable. If, therefore, the soil might be ground so that a representative sample could be obtained, the problem of getting more dependable results would be greatly simplified.

But in the grinding process a modification of the soil reaction undoubtedly takes place. The effect of this treatment on some Iowa soils has been recently recorded by Brown and Johnson.<sup>1</sup> Certain sandy soils were ground and tested for acidity, with the result that in all cases noted the lime requirement was reduced by grinding. In fact, some soils having a high lime requirement before treatment became basic thereafter. It is interesting to note that the finer the soil was ground the more basic it became, and also that the increase in basicity was roughly proportional to the amount of sand contained.

An explanation of this behavior might possibly lie in the assumption that the portions of the soils designated as "sand" were not sand in the chemical sense, but consisted of particles having a basic internal constitution, the surface of which may have become acid from exposure, or other causes. In this case the grinding, therefore, would be expected to increase the basicity. Since the finer particles of the soils are ordinarily composed of the more easily altered minerals it would not seem advisable to change their constitution more than necessary. There would also be no reason for grinding the fine particles which were already of the required fineness. Consequently, only those portions remaining upon the sieve were used in the grinding. Whether this procedure was followed, or the whole sample ground, is not stated in the work done at the Iowa Station.

If the sandy portions were silica, it appears that grinding would expose a greater portion of the acidic material and accordingly increase the acidity. It seems to be an established fact, as well, that for the same

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\* From the Soil Chemistry Laboratory, Rutgers College.

<sup>1</sup> Brown, P. E., and Johnson, H. W., The effect of grinding the soil on its reaction as determined by the Veitch method. *In* Jour. Amer. Soc. Agron., v. 7, no. 5, pp. 216-220, 1915.

material, that part which is in the finer state of division will usually manifest greater absorptive power. This physical factor strengthens our reasons for believing that the grinding of quartz sand would raise its "apparent acidity."

To test this point a sample of sand was examined for acidity both before and after grinding. The results recorded below seem to agree fully with the statements just made.

	Pounds CaO required per 3,500,000 pounds sand.
Quartz sand, unground .....	247.5
Quartz sand, ground to pass 100-mesh sieve .....	412.5
Increase due to grinding .....	165.0

Now if grinding free sand should increase its acidity there seems to be no reason why sand in soils (which in most sandy soils of New Jersey consists largely of silica and undecomposed silicates acidic in character) should not behave in a similar manner. With this in mind, a sandy soil was secured, put through a three-millimeter sieve, and that portion remaining on a 40-mesh screen was employed in the next determination. Most of this material was distinctly quartz and feldspathic mineral substance. A part of this was ground to pass an 80-mesh sieve, and the acidity of the ground and unground soil was then determined.

	Pounds CaO required per 3,500,000 pounds soil.
Sandy soil, unground .....	1320
Sandy soil, ground to pass 80-mesh sieve .....	1815
Increase due to grinding .....	495

Since this experiment served to corroborate the observation made with the quartz sand it was decided to test a number of soils to see if some, at least, would not react as indicated above. Samples of six different soils were obtained and prepared for determinations of acidity by passing them through a three-millimeter sieve. The soils were then further sieved through an 80-mesh sieve *and only those portions remaining upon it were ground*. When all was reduced to this fineness the ground material and that previously sieved were thoroughly mixed and the lime requirements of this and of the unground soil were determined.

The grinding was accomplished as before in a porcelain mortar. Any of the porcelain which may have been ground off did not seem to appreciably affect the reaction as determined in a blank. However, the estimation of the blank could not be made absolutely true, inasmuch as more porcelain would be taken off when the soil was being ground than when the mortar and pestle were used alone. It is, therefore, not impossible that a slight error was introduced at this point.

The results of the determinations made in each case are averaged and tabulated below :

POUNDS CaO REQUIRED PER 3,500,000 POUNDS SOIL.

Type	Soil No.1 Sass. Sandy Loam	Soil No.2 Norfolk Sand	Soil No.3 Colling- ton Sandy Loam	Soil No.4 Soil No.5 Soil No.6		
				Sassafrass	Gravelly	Loam
Sieved through 3 mm.	3135	1320	1485	Neutral	2970	3052.5
Sieved through 3 mm. and ground to pass 80-mesh	3630	1650	1732.5	412.5	3630	3217.5
Increase due to grinding	495	330	247.5	412.5	660	165.0

Here again there is shown to be an increase in acidity in all cases due to grinding. In one instance, that of Soil No. 4, a neutral soil became acid by this treatment. The increases observed do not appear to be much larger than could be accounted for by the increased surface of the sand particles, assuming them to be made up largely of silica.

There is some variation in the amounts of increases and it seemed worth while to determine if an approximate mechanical analysis would be of any value in explaining these differences.

MECHANICAL ANALYSIS OF SOILS.

Mesh	Soil No.1	Soil No.2	Soil No.3	Soil No.4	Soil No.5	Soil No.6
	%	%	%	%	%	%
3 mm.-20 meshes per inch	12	0	6	15	17	10
20-40 meshes per inch...	30	2	17	22	30	28
40-80 meshes per inch...	30	46	32	43	30	37
80 and above.....	28	52	45	20	23	25

An examination of this table does not offer much enlightenment upon the behavior observed. However, if we eliminate Soil No. 6, we get a correlation between the amount of acidity increase and the proportion of soil remaining above an 80-mesh sieve. Soils Nos. 2 and 3 then, show the lowest increases and show also the least amount of sand to be ground. Conversely, the largest increases are noted for Soils Nos. 1, 4, and 5, where the percentages of coarse particles are large. No explanation is offered for the slight alteration to Soil No. 6.

That diametrically opposite results were obtained at the Iowa station may point to a very interesting difference in the respective soil types. In no case, however, was the increase in acidity in New Jersey soils nearly as marked as the increase in basicity in Iowa soils, but the grinding has a distinct effect in either instance. Both observations point to the fact that soils should not be ground for acidity determinations.



## SUMMARY.

The experiments outlined above tend to indicate that:

1. Soils should not be ground if used for determination of lime requirement by the Veitch method;
2. Grinding sandy soils of New Jersey increases their acidity, instead of decreasing it, according to the method employed.

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## THE ACTINOMYCES OF THE SOIL.\*

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The study of soil microorganisms has attracted the attention of many investigators, and great advance has been made in the study of bacteria, fungi, and protozoa. Very little attention, however, has been paid to the actinomycetes as a group of soil organisms. The present work has been undertaken with the purpose of demonstrating the occurrence of actinomycetes in different soil types, at different depths, and under different cultural and climatic conditions. An attempt has been made to secure a knowledge of the physiological activities of these organisms and their possible part in soil fertility.

During the past forty years about forty types of actinomycetes have been studied under different names and under varied environmental conditions. Most of these descriptions are so incomplete that it was found impossible to identify many of the organisms at hand with those previously studied. Almost all former investigations have been undertaken from a pathological standpoint, and the descriptions have been adapted to that purpose. With one exception, the studies heretofore made on soil actinomycetes have been limited to a very few representatives of this group.

The present paper records an attempt to study the occurrence of actinomycetes in the soil, and to classify them according to their morphological and physiological characters; it is necessarily incomplete, inasmuch as the sources of literature for study and identification are so limited and the field of investigation is so large.

### HISTORICAL.

Cohn (5), in 1875, was the first to study an actinomycete, under the name of "*Streptothrix*" Foesteri. Bollinger (2) found one in an "actinomycose" swelling of cattle, and named it *Actinomycetes* for its radiating form. Rossi-Doria (19) studied *Streptothrix alba*, *Str. nigra* (*Str. Foes-*

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teri), *Str. albido-flava*, *Str. violacea*, *Str. carnea* and *Str. aurentiaca*. Gasperini (9) made an important contribution to the knowledge of actinomyces by his work on *Act. albus*, *Act. sulphureus*, *Act. luteo-roseus*, *Act. asteroides*, *Act. carneus* and *Act. aurentiaca*. More work followed: names were mixed and the terms "Actinomyces," "Streptothrix," "Cladotrix" and "Oöspora" were often interchanged, all of them meaning the same type. Petruschky (18) united all fine mycelial, unseptate fungi into the family "*Trichomycetes*," and divided this into four groups : (1) Actinomyces, those forming a radiating growth when parasitic in animal tissues, (2) Streptothrix, having an abundant true branching, wavy or curly growth, late fragmentation and formation of conidial chains, which serve as organs for multiplication, (3) Cladotrix, having false branching, quick fragmentation and therefore bacillary character of old cultures, (4) Leptothrix, never shows true branching, but stiff, little curved hyphae on which no organs of division can ever be detected.

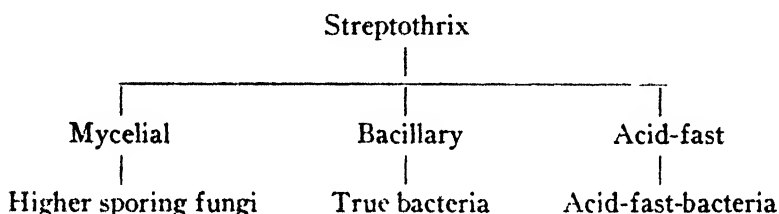
Lehmann and Newmann (14) consider the actinomyces a special group, which stands between the Hyphomycetes and Schizomycetes; related to the latter by their slender hyphae and protoplasmic properties, and to the former by the branching formation of aerial hyphae with conidia-like structures. They are defined as "delicately threaded organisms, with true branching, in part very abundantly ramifying mycelium, partly with the formation of conidia. There is a tendency to the formation of clubs or knobs at the ends of threads." This family is divided into two groups. Group I contains the corynebacteria (L & N), which are "slender, often somewhat bent rods, usually having a tendency toward a clubbed swelling at the ends, branches rarely observed in young cultures, easily broken off; always non-motile, conidia never found"; and Mycobacteria (L & N), with "clubbed swellings rare in cultures, in tissues somewhat more common, staining with difficulty or not at all." Group II, the Actinomyces, are described as follows by Hartz: "Mycelial threads long, thin; extending or winding; dividing without partitions, with delicate sheaths and true branching; many species separate from the hyphae rows of short spores (conidia) which, whitish and mold-like, project upward above the solid nutrient substratum. Motility sometimes manifested. Almost all varieties emit a musty odor."

Sanfelice (21) very ably pointed out the faults in Petruschky's classification. He showed that actinomyces are, according to morphology and properties as revealed in culture, true and proper Streptothrices. The peculiarity, which seemed of so much importance to Petruschky that he separated the Actinomyces from the Streptothrix types, is only a specific property. The term "Actinomyces" as understood by Sanfelice, and by Lehmann and Newmann, will be used by the authors, since this term alone

can be applied to the great mass of the soil microörganisms which are discussed in this paper.

Musgrave (17) and others used the name *Streptothrix* for the genus. They define *Streptothrix* "as branching filamentous organisms, which develop into colonies made up of organisms and their transformation products. Terminal hyphae may or may not be radial, may or may not have clubs. This group is in general gram positive and many are acid fast." Foulerton (7) describes *Streptothrix* as "a tangled mass of branching mycelium. The mycelial stage is followed by segmentation and fragmentation, producing bacillary forms; and in artificial media by chain sporulation."

Claypole (4) gives the following tentative outline for the development of the microörganisms from the *Streptothrix* group:



She says, "The limits of species variation of the *Streptothrices* are neither set nor well known. The cause of confusion and for diverse opinions and practices lies in the extreme morphological and biological variability of these fungi. Some strains grow feebly on all media; some only on special media, and some apparently cannot be cultivated. Much variation is to be found in the morphology of any given organism. It changes its appearance with differing culture media. The well known granules or 'Drusen,' a branched mycelial mass, fragmentations into apparent bacilli and cocci, true spores as well as the minute structures left after chain sporulation, may be found in the life history of one species. It would seem biologically more reasonable to look upon this group of *Streptothrices* with their variable morphology and close relationships, as representing the ancestral type that gave both the higher fungi and the true bacteria and not as being themselves, higher bacteria."

Rullmann (20), the first to study the actinomyces in the soil, concludes that *Act. odorifer* causes the soil odor, which it also forms on media containing carbohydrates, but that it has no nitrifying ability.

Beijerinck (1) studied the existence of actinomyces in nature and their ability to form "quinone," an oxidizing agent. He found actinomyces in garden soil even at a depth of one meter, and in dune sand as deep as two meters; he found them also on the roots of many plants. He states that they inhabit the outside cells of the plants as saprophytes,

not as parasites. They are omniverous. They can live on media free from combined nitrogen, which they get from the air or distilled water (not atmospheric nitrogen), so small is their nitrogen requirement. They must play an important, if not a dominant, part in humus formation. He brought out the peculiar property of the actinomyces, namely, their power to reduce nitrates to nitrites without causing much loss of nitrogen. He also found that subsoils, though containing fewer numbers of actinomyces than the surface soil, are relatively richer in these than in other microorganisms. The only types studied by Beijerinck were *Act. albus* and *Act. chromogenus*.

Hiltner and Störmer (12) found that in the spring actinomyces form 20 per cent of the total bacterial numbers in the soil, while in the fall they form 30 per cent.

Fousek (8) found in the fall a greater percentage of actinomyces than in the spring. He found them to form 20 to 30 per cent of the organisms in loam soils, 8 to 15 per cent in clay soils, and 7 to 10 per cent in sands. Fallow soils contained larger numbers than cultivated soils. The actinomyces assimilate nitrates, ammonia and amido nitrogen, and form ammonia from organic substances. Nitrates are reduced to nitrites; free nitrogen is not assimilated. He finds *Act. albus* and *Act. chromogenus* to be predominant among the soil actinomyces.

Hagem (10) isolated four actinomyces from the soil, but from his short description of the macroscopic appearance of the organisms, one can hardly get a true idea as to which species he really had.

Münter (16) isolated seven organisms from different soils: *Act. odorifer*, *Act. chromogenus*, *Act. albus* I and II, and three more organisms which he terms *Act. S-a*, *Act. S-b*, and *Act. S-c*. He finds that these organisms can assimilate sugar and organic salts; and that organic substances have a strong influence on pigment production. The organisms are very sensitive to acids and alkalies. All of them liquefied gelatin, with or without the production of a brown pigment.

Conn (6) found that actinomyces may make up as much as 40 per cent of the soil bacteria.

The most complete work on the actinomyces of the soil is that of Krainsky (13), who has given a full description of eighteen well characterized and defined species of soil actinomyces. The organisms have been studied on different media and rather complete morphological and physiological qualities are recorded. All of them reduce nitrates to nitrites to a greater or less extent. Krainsky studied the production of enzymes, as did Münter and several others. Some of Krainsky's organisms are reported as strong cellulose destroyers. On allowing plates of calcium malate agar to incubate for thirty days he found 20,800 colonies of actino-

myces per gram of soil, which was 30 per cent of the total number of organisms developing on this medium. The upper soil layer was found to be poorer in fast growing forms than the soil at the depth of fifty centimeters. Krainsky concludes that the actinomyces play an important part in the decomposition and humification of plant remains in the soil.

## EXPERIMENTAL.

### I. METHODS OF STUDY.

#### 1. *Soils used.*

Seven soils have been considered in this work. They represent different types, from several localities and under differing climatic and cultural conditions. The principal purpose was to determine whether there are any so-called soil actinomyces, whether organisms isolated from one locality are found in another, and whether there is a constancy in the occurrence of the particular species. Three soils from the eastern Los Angeles County, California, were among those used. The samples from these represent a composite of the surface eight inches. They are: (1) an upland, residual, loam unirrigated and cropped for grain and hay, which will be designated as "upland soil"; (2) a heavy adobe soil, irrigated, from an orange orchard, termed "adobe soil"; (3) a sandy loam, irrigated, also from an orange orchard, termed "California loam." A fourth soil was secured from the experimental farm of the Oregon Agricultural College at Corvallis, Oregon. This is an adobe type and has been cropped to legumes and small grains, termed "Oregon adobe." The three remaining types were taken from the experimental grounds of the New Jersey Agricultural Experiment Station at New Brunswick, N. J., (5) a Sassafras sandy loam, heavily manured every year, under garden crops, and termed "garden soil"; (6) a Sassafras sandy loam unmanured for the past twenty years, under orchard, termed "orchard soil"; and (7) a heavy clay soil, under permanent meadow, termed "meadow soil." Samples from the last three soils have been taken at depths of 1, 4, 8, 12, 20 and 30 inches. All sampling has been done under sterile conditions, and in the subsequent handling and plating the usual bacteriological precautions against air or other contaminations have been observed. The New Jersey soils were plated out within a few minutes after the samples were taken. In the case of the Oregon and California soils the necessary time for shipment of course elapsed between sampling and plating.

#### 2. *Media used.*

Most investigators have used beef extract agar and gelatin for the study of actinomyces. As was pointed out before, the actinomyces will grow readily on any medium containing enough carbohydrates. Their nitrogen requirements are very small. Beef extract agar and gelatin are

not suitable media for the culture of actinomyces, because, first, they are rich in nitrogen and for this reason do not bring out the characteristic colors of the organisms, aerial mycelium is not readily formed, and when it is produced, is of a chalky color; furthermore, most species tend to produce colonies more or less white in color accompanied by a brown pigment in the substratum. Second: These media are not constant in composition, and the growth and color of the actinomyces, two of the most important factors in their differentiation, are very sensitive to change in the composition of the medium. The above probably accounts for the fact that early investigators reported one *Act. albus* and one *Act. chromogenus* in the soil.

Krainsky has used in his work a calcium malate agar and several other synthetic media. For this, as in any bacteriological work, media of constant chemical composition are desirable. As actinomyces may change considerably in color production and character of growth from the mere process of transferring several times on the same medium, it can be readily seen that different media, as well as those of varying composition, would give very incomparable results.

Brown's (3) albumen agar, slightly modified, has been used for the isolation of the organisms from the soil. Suitable dilutions, varying from 1:200,000 for the surface soils to as low as 1:10,000 for the deeper subsoils, were used. The plates were allowed to incubate for 14 days at 22° C. Counts were then made and the actinomyces transferred to Czapek's solution agar. On this latter medium macroscopic and microscopic studies of all the organisms were made. Each organism was also studied on potato plugs (incubated at 30° C.) and on 15 per cent gelatin, in distilled water (incubated at 15° to 16° C.). Some species were studied on Czapek's solution, where a characteristic growth is produced; and on 1 per cent dextrose broth, for gas production. A few were studied on beef extract agar, mannite agar, and several other media.

All the actinomyces studied liquefy gelatin. They seem able to get all necessary food from the pure gelatin in distilled water, decomposing the gelatin in all probability by means of an enzyme. The various species show marked differences in the rapidity with which they liquefy gelatin. Some form a liquid ring of 1 to 2 cm. diameter in three days; others hardly form a liquefied circle of 2 mm. diameter in ten days. There is also a difference in color production on gelatin. In this connection the actinomyces could be divided into two groups: those that do not produce any color on gelatin, the liquefied portion remaining pure white; and those that produce a pigment (usually brown) in and around the liquefied portion. The depth of the pigment varies somewhat in the different species. Some of the organisms produce aerial mycelium on the gelatin. This appears to be characteristic of the particular species.

### 3. Numbers of Actinomyces in the Soil, and Their Relation to Numbers of Bacteria.

One of the authors (23) has pointed out the fact that, though the numbers of actinomyces decrease with soil depth, their numbers, relative to those of bacteria and fungi, greatly increase. At the depth of one inch the actinomyces made up from 7.3 to 12.1 per cent of the total number of microorganisms; at the depth of 30 inches they constituted 52.7 to 83.6 per cent of the total numbers.

Additional data are presented in the following tables.

TABLE I.  
BACTERIA AND ACTINOMYCES IN NEW JERSEY SOILS.

Soil Depth in inches	Garden Soil				Orchard Soil				Meadow Soil			
	Bacteria		Actinomyces		Bacteria		Actinomyces		Bacteria		Actinomyces	
	Numbers	%	Numbers	%	Numbers	%	Numbers	%	Numbers	%	Numbers	%
SEPTEMBER 15, 1915.												
1	7,870,000	93.7	533,000	6.3	7,000,000	92.9	533,000	7.1	8,600,000	90.9	867,000	9.1
4	6,400,000	87.3	933,000	12.7	6,200,000	88.6	800,000	11.4	7,200,000	85.0	1,267,000	15.0
8	3,670,000	93.2	267,000	6.8	2,930,000	92.6	233,000	7.4	3,933,000	86.8	600,000	13.2
12	1,867,000	93.0	140,000	7.0	1,353,000	90.6	140,000	9.4	767,000	81.6	173,000	18.4
20	320,000	62.4	193,000	37.6	140,000	52.6	126,000	47.4	320,000	63.2	187,000	36.8
30	113,000	31.4	247,000	68.6	53,000	29.6	126,000	70.4	153,000	50.0	153,000	50.0
NOVEMBER 2, 1915.												
1	4,700,000	87.0	700,000	13.0	4,600,000	88.5	600,000	11.5	15,400,000	92.2	1,300,000	7.8
4	4,500,000	85.0	800,000	15.0	4,500,000	77.6	1,300,000	22.4	7,000,000	86.4	1,100,000	13.6
8	3,500,000	74.5	1,200,000	25.5	1,560,000	76.5	480,000	23.5	1,710,000	79.9	430,000	20.1
12	720,000	62.1	440,000	37.9	670,000	59.0	470,000	41.0	1,040,000	79.4	270,000	20.6
20	210,000	12.0	290,000	58.0	130,000	19.4	540,000	80.6	690,000	67.0	340,000	33.0
30	160,000	24.0	510,000	76.0	90,000	16.4	460,000	83.6	160,000	44.6	200,000	55.6
NOVEMBER 30, 1915.												
1	5,300,000	85.5	900,000	14.5	4,800,000	86.6	700,000	13.4	8,100,000	93.7	550,000	6.3
4	4,300,000	84.3	800,000	15.7	3,200,000	82.0	700,000	18.0	4,500,000	86.5	700,000	13.5
8	3,600,000	75.0	1,200,000	25.0	1,800,000	81.8	400,000	18.2	1,700,000	70.8	700,000	29.2
12	725,000	82.0	160,000	18.0	680,000	74.7	230,000	25.3	750,000	85.2	130,000	14.8
20	160,000	39.0	250,000	61.0	330,000	58.9	230,000	41.1	30,000	33.3	60,000	66.7
30	90,000	35.6	170,000	64.4	250,000	51.0	240,000	49.0	50,000	50.0	5,000	50.0

GENERAL AVERAGE FOR ALL THREE SOILS.

Soil Depth	Bacteria		Actinomyces	
	Numbers	%	Numbers	%
1 inch	7,340,000	90.8	743,000	9.2
4 inches	5,300,000	85.0	933,000	15.0
8 inches	2,710,000	81.6	612,000	18.4
12 inches	950,000	79.9	239,000	20.1
20 inches	259,000	51.3	246,000	48.7
30 inches	124,000	34.6	240,000	65.6



The data presented in Table I bear out well the observations of previous investigators, in regard to the distribution of actinomyces in the soil and their numbers, relative to those of bacteria.

The numbers of both actinomyces and bacteria are greatest in the surface soil, but while the bacteria decrease rapidly below a depth of four inches, the numbers of actinomyces are practically constant at depths from 8 to 30 inches. This point is graphically brought out in Fig. 1, where the average percentages of bacteria and actinomyces from Table I are plotted.

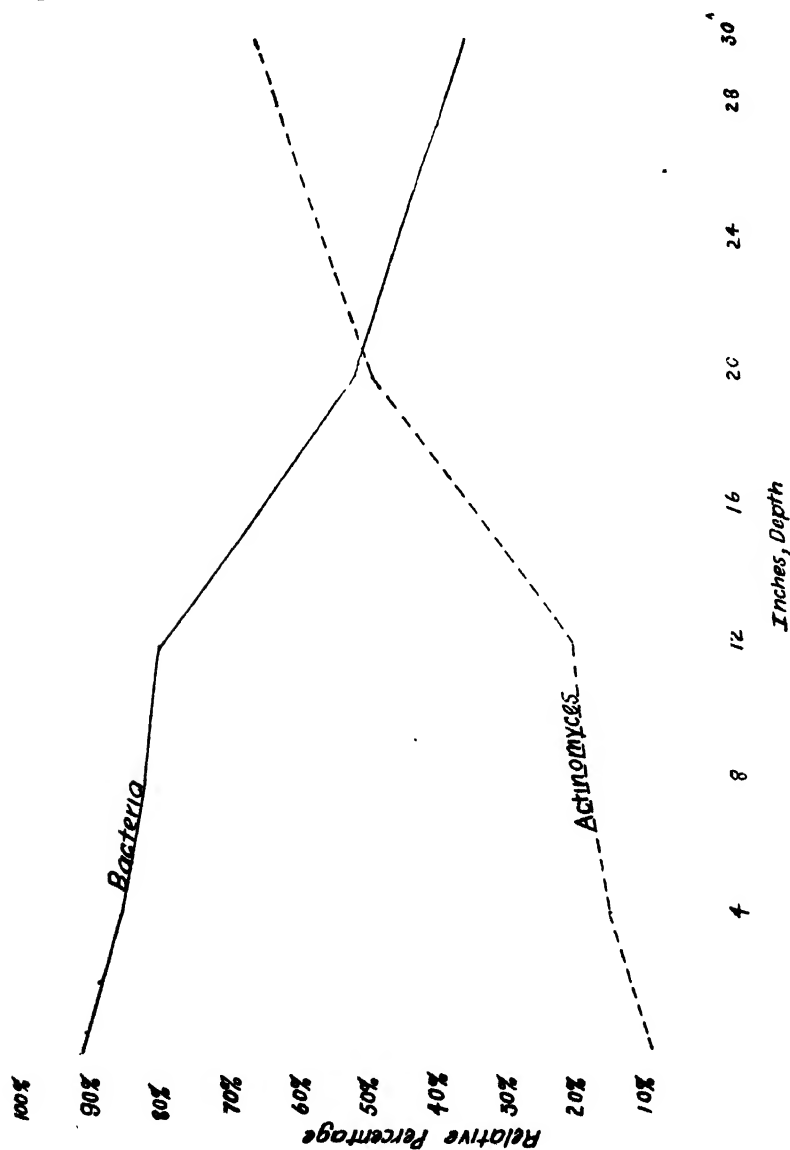


Figure 1—Relative percentages of Bacteria and Actinomyces at Different Soil Depths.

TABLE II.  
BACTERIA AND ACTINOMYCES IN OREGON SOIL.

Depth	Bacteria		Actinomyces	
	Numbers	Per cent	Numbers	Per cent
0—2 inches .....	13,100,000	84.6	2,400,000	15.4
3—5 inches .....	10,600,000	63.1	6,200,000	36.9
10—14 inches .....	2,960,000	63.8	1,680,000	36.2
18—22 inches .....	680,000	48.6	720,000	51.4
28—32 inches .....	800,000	64.6	440,000	35.4

Table II presents the numbers and percentages of actinomyces and bacteria in the "Oregon adobe" soil. A close resemblance is seen between the percentages of bacteria and of actinomyces when the figures are compared with those in Table I. This is particularly true of the "meadow soil" and the "Oregon adobe." As the climatic conditions in the two places are very similar and the two soils have much in common, e. g., fine texture, large water capacity and a high water table, the similarity in results was to be expected.

TABLE III.  
BACTERIA AND ACTINOMYCES IN CALIFORNIA SOILS.

Soil	Bacteria		Actinomyces	
	Numbers	Per cent	Numbers	Per cent
Upland (Sample I) .....	1,935,000	80.4	380,000	19.6
Upland (Sample V) .....	2,310,000	55.0	1,890,000	45.0
Upland (Sample VI) .....	2,420,000	62.7	1,445,000	37.3
Sandy Loam .....	6,010,000	80.8	1,430,000	19.2
Adobe .....	3,620,000	78.0	800,000	22.0

Table III gives similar data obtained from the southern California soils. As was noted before, these samples were a composite of the surface soil to a depth of 8 inches. A comparison of these figures obtained from semi-arid soils with those from humid soils brings out striking differences. In the surface eight inches of the New Jersey soils studied, actinomyces make up an average of 14.2 per cent of the micro-flora, exclusive of the higher fungi; while in the California soils this average is 28.6 per cent. This seems to indicate that semi-arid surface soils are relatively much richer in actinomyces than are those of humid regions.

### MORPHOLOGY OF THE ACTINOMYCES.

The actinomyces grow on artificial media in the form of small, slowly developing, usually round colonies, which are partly submerged and partly aerial. Most of them usually begin to develop from the bottom of the plate, slowly pushing upward their rounded, glossy surface, until, on reaching the surface of the medium, they usually become covered with an aerial mycelium, which reminds one of lower spore-forming fungi. The aerial mycelium forms aerial spores which serve for reproduction. This

typical differentiation between the substratum and aerial growth, and the characteristic coloration of the colony and the aerial mycelium, which is of great importance as a basis for the separation of types, is had only under favorable conditions: in the presence of air and the proper medium. The colony is not smeary as in the case of most bacteria; the growth is solid and discrete. It can easily be lifted from the plate with a platinum needle, without breaking the colony. Placing the plate under the microscope, one can easily tell whether the organism is an actinomyces or bacterium, by the radiation of the thin hyphae from the colony into the medium. The surface of the growth is either smooth or undulated, ridged, folded, and even exfoliated, the colony having sometimes a lichenoid appearance. The edge is usually entire and filamentous. The substratum growth consists of very fine, branching mycelium, reminding one of bacterial structure, as there is no differentiation among wall, protoplasm, and cell sap. Septa are not formed and the younger branches are formed irregularly on the older ones. The aerial mycelium may cover only the central part of the colony, leaving a free margin, but very often the whole colony is covered with aerial mycelium. The latter usually consists of thicker hyphae than the substratum mycelium. They often end in club-like structures, or in spirals of different curvatures. Some of the species form spirals abundantly, the latter being generally characteristic of the type. The aerial hyphae break down into spherical, oval, or rod-shaped spores, of different sizes, and which are often united in pairs or in chains. Some organisms produce spores at an early stage, some only after they are several weeks old, some species do not form spores at all. The color of the aerial mycelium is characteristic of the species; sometimes it may be two-colored, at first white, only later developing the characteristic color. The color of the mycelium changes with the different media used, being influenced by the carbon and the nitrogen source. The colonies themselves are either colorless, or yellow, red, green, brown, black, blue, or of some other color. Many organisms produce a soluble pigment, which colors the medium, and this is also characteristic of the species. The three "violaceus" types produce a violet, blue or dark pigment on Czapeck's agar, while on potato only the "violaceus-ruber" produces the blue pigment, the other two species producing no pigment at all.

The growth on gelatin has been described before. Krainsky (13) found only the *Act. citreus* to produce an aerial mycelium on the bouillon gelatin; the present work has shown that many species produce an aerial mycelium on pure gelatin in distilled water, though not so readily as on Czapeck's agar.

Liquid media give characteristic growths with different species. All the organisms could be classified into four groups by their growth in

Czapeck's solution, as follows: those producing (1) a flaky growth on the bottom of the flask; (2) flaky growth all through the medium; (3) individual, well defined colonies all through the medium; (4) combined flaky growth on the bottom and colony formation through the medium. The character of colony formation may be specific. With some species the colonies are attached to the wall of the flask; others form colonies which always float free. Colonies may unite in masses on the surface of the liquid or at the bottom of the flask.

Dextrose broth (1 per cent) in fermentation tubes was inoculated with a considerable number of the species studied in order to determine their gas production. Not only was no gas produced in any case, but every organism failed to grow in the closed end of the tube. This fact shows that the actinomyces are not preferential anaerobes. This has already been pointed out by Beijerinck, who classed them as facultative anaerobes.

An interesting point in the growth of some actinomyces is the production of rings in the colony. This is especially prevalent on poor media. The ring formation may take place in the vegetative portion of the colony as well as in the aerial mycelium. So far no adequate explanation has been found for this phenomenon.

#### CLASSIFICATION OF THE ACTINOMYCES.

Most of the work on the identification of the actinomyces has been undertaken from the pathological point of view. Different media have been used and different characters recorded. The result is, that with few exceptions, it is impossible to identify soil organisms with those that have been described before. For example: "*Act. chromogenus*" was supposed to be a well defined organism, yet Krainsky (13) had four representatives of the group. The authors have eight "chromogenus" types, each with such well defined characters as to make it almost impossible to classify them as one species.

The only work that could be satisfactorily used for the classification of the organisms at hand was that of Krainsky (13). Sanfelice (21) has classified the actinomyces into three groups: (1) *albus*, (2) *flavus*, (3) *violaceus*. This grouping, based merely on the color of the colony, is purely arbitrary, because, as has been pointed out, the color varies with the media used. Krainsky classifies the actinomyces into: (1) the Macro-group, which appear early on the plates, form large colonies, and bear oval or spherical conidia, (2) the Micro-group, appearing late, in small colonies, and producing spherical conidia. They are strong cellulose decomposers. (In this group belong the organisms producing violet and yellow colonies.)

This method of grouping is far from perfect. The size of the colony

and the time of its appearance on the plate depend not only on the organism itself, but on the media used and the incubation temperature. Also, the fact that both groups may produce spherical conidia is confusing.

In the present work it was thought advisable to describe the organisms as to their characteristic growth on different media. When sufficient material has accumulated it may be possible to work out a system of classification having for its foundation the more stable and important characters of the organisms.

While the grouping of the organisms according to the rapidity with which they liquefy gelatin and according to their production or non-production of pigment in this medium, does not meet all the objections to such a classification, it is believed by the authors that it offers a starting point for identification. The term "rapid liquefaction" is here applied to liquefaction of 15 per cent gelatin in distilled water, in three days at 15° to 17° C.

Over one hundred organisms have been isolated from the soil. These represent 30 species described in the present article. Several organisms have produced such scanty and uncharacteristic growth on the media used that it has been thought advisable to keep them under observation for a longer period, in the hope of detecting important stages in their life history.

#### DESCRIPTION OF THE *ACTINOMYCES* ISOLATED FROM THE SOIL.

##### *Act. violaceus*, n. sp.

*Czapeck's Agar.* Colony at first colorless, turning red and blue, colors seen very clearly in the reverse. The red color is soon absorbed by the profuse production of a cyanine blue (Rdg.\* ix-51-m) pigment, which diffuses through the medium. Colonies 1 to 3 mm. in diameter, showing rapid growth and formation of zones. Surface of colony smooth with a narrow, entire, white margin. Aerial mycelium appears at an early stage of the colony, at first white, then turning to mouse-gray (Rdg. li-15'''). It has a silvery appearance due to the drops of water exuded upon the surface. Odor present, but weak. Microscopically two kinds of mycelium could be determined: the substratum mycelium consisting of fine closely branched filaments of a red to blue color; and the aerial growth consisting of thicker, straight filaments with very little branching at the edge of the colony, but more branching in the centre. Numerous spirals are found in the aerial mycelium. These as well as the hyphae break up readily into oval to rod-shaped conidia, 0.8 to 1.5 x 0.7 to 1.0 $\mu$ .

*Gelatin.* Spreading, dense, colorless growth, with an early production

\* This abbreviation throughout this paper refers to Ridgeway, "Color Standards and Nomenclature."

of a white aerial mycelium, underlaid by a pinkish coloration. Gelatin around the colony slowly liquefied, remaining clear.

*Potato plug.* After 36 to 48 hours, growth appears as a mass of well defined, round colonies, 1 mm. in diameter. White aerial mycelium is produced at an early date. Color of plug is at first unchanged, but after 4 to 5 days red and blue pigments are produced, either of them predominating; these also slightly color the white aerial mycelium.

*Czapeck's solution.* Growth consists of a flaky mass on the bottom of the flask, with numerous, small, round colonies all through the medium.

*Glucose solution.* A solid growth in the form of a grayish-white ring is formed on the surface, close to the side of the flask, with no growth through the medium. White to gray aerial mycelium appears at an early stage.

*Hab.* Isolated several times from the California adobe soil. Herbarium Nos. 8 and 44.

*Act. violaceus-Caeseri*, n. sp.

*Czapeck's Agar.* Growth very slow, consisting of gray colonies, 2 to 3 mm. in diameter. Surface of colony glossy and much folded. Aerial mycelium produced very late; it is pure white, with no shading into gray. A plum-purple (Rdg. xxiv-57-m) pigment is produced at an early stage, and gradually diffuses all through the medium. Pigment is much darker than that produced by *violaceus-ruber*, and no red tinge is ever observed. Medium becomes so dark from the diffused pigment as to be black by reflected light. Weak odor is given off. Microscopical examination shows the mycelium to consist of fine filaments, with the production of numerous open spirals in the aerial mycelium. Conidia could not be demonstrated.

*Gelatin.* Growth very small, with no aerial mycelium produced. Gelatin around the colony is rapidly liquefied, with no change in color.

*Potato plug.* Growth consisting of small yellowish colonies, which develop very slowly. Little aerial mycelium seen even after cultures are 20 days old. The color of the potato is not changed. No pigment is produced.

*Czapeck's solution.* Small flaky growth on the bottom of the flask. None through medium. All medium is colored blue at an early date. This is the only organism which colored deeply the Czapeck's solution.

*Glucose solution.* Thin, flaky growth on bottom of the flask, with none through medium.

*Hab.* Isolated once from the upland California soil. Herbarium No. 31.

*Act. violaceus-niger*, n. sp.

*Czapeck's Agar.* Colony at first dark gray, turning almost black. 2 to 4 mm. in diameter. Surface glossy, much folded with a very thin,

gray margin. A white to gray aerial mycelium is produced after the colony has well developed. A bluish black pigment is produced at a later stage of its growth. The pigment slowly dissolves in the medium, turning almost black. Odor fairly strong. Microscopically two types of mycelium were found: the thin, branching filaments of the substratum, and the thick filaments of the aerial mycelium. The aerial mycelium fragments not very rapidly, producing a few conidia, spherical and oval, 1.2 to 2.3 x 1.2 to 1.5 $\mu$ . These often occur in chains.

*Gelatin.* Gray growth on gelatin, with no production of aerial mycelium. Gelatin around colony rapidly liquefied, but without any change in color.

*Potato plug.* Growth at first very slight, but after 48 hours it develops in a yellowish-gray continuous thick smear, which turns brown at a later date. White aerial mycelium covers the growth late. Plug is not discolored.

*Czapeck's solution.* Colonies large, 2 to 3 mm. in diameter, appearing at the bottom and surface of the solution, but none throughout the medium. The colonies are bluish in color, with a regular margin. Medium is not colored.

*Hab.* Isolated once from the upland California soil. Herbarium No. 39.

*Act. erithrochromogenus*, Krainsky.

*Czapeck's Agar.* Colonies small, round, 2 to 3 mm. in diameter, with a slightly cut margin. Color of colony is at first tawny-olive (Rdg. xxix-17"-i) to a buffy-brown (Rdg. xl-17"-i). Surface smooth at first, then lichnoid. White to gray aerial mycelium found at a later stage: this does not cover the whole surface, appearing only at separate parts of it. Reverse of colony dark. The production of soluble brown pigment at an early stage of its growth is characteristic of this organism as well as of all the other chromogenes species. The substratum mycelium consists of fine filaments with little branching. Aerial mycelium does not show any distinguishable structure. Conidia are abundant and fairly large, rod-shaped, 1.5 to 2.4 x 1.1 to 1.4 $\mu$ . A strong odor can be easily detected.

*Gelatin.* After several days of growth, each colony is found at the bottom of a small pit. Centre of colony is yellow, edge hyaline. The radial mycelium extends into the unliquefied portion of the gelatin. Slight white aerial mycelium is found on the surface of the older colonies. Gelatin around the colony is liquefied very slowly and is colored brown.

*Potato plug.* Growth consisting of small individual colonies, forming a continuous streak all over the plug. Colonies are at first yellow-gray, becoming with age dirty-gray and glossy in appearance. Aerial mycelium not formed readily. Plug is darkened.

*Czapeck's solution.* Growth consists of individual, round, brown colonies 1. to 1.5 mm. in diameter all through liquid and on surface.

*Glucose solution.* Very little growth takes place.

*Hab.* Isolated from the upland and adobe California soils. Herbarium Nos. 17 and 18.

*Act. diastato-chromogenus*, Krainsky.

*Czapeck's Agar.* The description of this organism coincides very closely with that given by Krainsky for the organism under the same name. Colonies fairly large (3 to 5 mm.), at first colorless, then becoming brownish. Gray aerial mycelium colors all the colony at an early stage without leaving any free margin. Small drops of water are exuded upon the surface. Reverse of colony is brown, and a light brown pigment is produced dissolving through the medium. Odor present, but weak. Microscopically, the aerial mycelium is found to consist of long filaments, with very little branching. These break up readily into rod-shaped conidia,  $1.5 \text{ to } 1.8 \times 1. \text{ to } 1.2\mu$ .

*Gelatin.* Gelatin is rapidly liquefied with the production of a brown color.

*Potato plug.* Growth light gray turning brownish, in one continuous streak, with several small separate colonies (1 mm.). White aerial mycelium appears at an early date and covers the whole growth. Potato is blackened.

*Czapeck's solution.* Flaky growth on the bottom of the liquid, and a pellicle on the surface. No growth through the medium.

*Glucose solution.* Flaky growth on the bottom of the liquid, and a ring of individual colonies 1 to 2 mm. in diameter on the surface of the liquid close to the glass of the flask.

*Hab.* Isolated twice from the California adobe soil. Herbarium Nos. 41 and 47.

*Act. purpeo-chromogenus*, n. sp.

*Czapeck's Agar.* Colonies small, 0.5 to 1.5 mm. in diameter, developing very slowly. They are brown in color with a brown to black reverse. Surface of colony is glossy, and raised in the centre. A brown soluble pigment is produced, which shows a distinct purple tinge, when viewed by transmitted light. Aerial mycelium is formed later. When culture is over 4 weeks old a brownish purple to black surface mycelium is formed. Margin of colony is waxy-yellow in color and lichnoid in appearance. No odor could be detected. Microscopically, no difference could be seen between the substratum and surface mycelium, a condition which suggested the question whether or not the latter could be called aerial mycelium. No conidia could be found. Substratum mycelium seems to break up into spherical non-staining bodies,  $.75 \text{ to } 1.\mu$  in diameter (oidia?).



*Gelatin.* Growth very slow. Liquefaction of the gelatin is slow with the production of a brownish pigment only at a late period.

*Potato plug.* No growth on potato in 2 to 3 days at 30° C. Only after 10 days the colonies were found to be very small, orange colored, grouping in a bead-like fashion. Colonies become dark brown with age. Potato slightly colored brown.

*Czapeck's solution.* Flaky growth on the bottom of the flask.

*Hab.* Isolated once from the California adobe soil. Herbarium No. 49.

*Act. viridochromogenus* (?), Krainsky.

*Czapeck's Agar.* Colony 2 to 8 mm. in diameter, at first yellowish-gray, slightly raised above the substratum. Surface glossy, granular, at first yellowish-brown and finally becoming dark green in color. White aerial mycelium appears first at the edge of the colony, rapidly advancing toward the centre, until finally the whole colony is covered with a white-gray aerial mycelium. Margin of colony is colorless, regular. Reverse of colony is colored at first yellowish-gray, later becoming dark brown. A dark brown to black pigment is produced which dissolves through the medium. Microscopically, the aerial mycelium is found to consist of a dense mass of filaments with little branching. The filaments fragment easily into long pieces, and these usually break up into oval-shaped spores, 1.2 to 2 x 0.75 to 1.1 $\mu$ . Odor present but not very pronounced.

*Gelatin.* Gelatin rapidly liquefied with the production of a brown pigment.

*Potato plug.* Growth much folded, continuous streak, at first grayish yellow to green, but finally becoming dark green. White aerial mycelium is produced. Potato becomes black.

This organism seems to coincide with Krainsky's "viridochromogenus," though it differs from it in some details.

*Hab.* Isolated once from garden soil at a depth of 8 inches. Herbarium No. 61.

*Act. chromogenus* group.

Besides the four species of "chromogenus" previously described, several more organisms have been isolated, which show the characteristic features of the "chromogenus." These cannot be classified as one species, because they show distinct characters from one another. All of them are characterized by a colorless colony, changing later to different shades of brown. Surface mycelium is either absent or produced when culture is over 2 to 3 weeks old, and is of a brown to black color, hardly differentiated from the surface of the colony. Some organisms may produce small white tufts at a late stage. Reverse of colony is brown to black.

A brownish pigment diffuses through the substratum. The colonies are hard, a condition due probably to the production of quinone, which is characteristic of all the "chromogenus" species, as was pointed out by Beijerinck. Gelatin is liquefied at first, but the liquefaction does not advance far; the hardening up is also probably due to the quinone production. A brown pigment diffuses through the unliquefied portion of the gelatin. These cultures have shown the same general characteristics as the *Act. chromogenus* isolated from potato scab, a culture of which was borrowed from the plant pathology department of the New Jersey Agricultural Experiment Station.

STRAIN No. 1. Colonies 3 to 5 mm. in diameter. Surface smooth, dry, with lichnoid margin. Color yellow becoming overlaid by a dry, thin, black sheet, with the production of white aerial mycelium in centre of colony at a late stage. Tendency to grow in individual colonies, and not to form a solid streak. General outline of colony is yellow to black. Reverse dark brown to almost black, medium colored brown. Microscopically, aerial mycelium was found to be either lacking or very scarce. Surface mycelium consists of thick hyphae, but no clubs observed. Conidia few, oval to rod-shaped, 1.2 to 1.8 x 1.  $\mu$ .

*Hab.* California upland soil. Herbarium Nos. 40 and 48.

STRAIN No. 2. Colonies 3 to 5 mm. in diameter, colorless at first, becoming later gray. Surface glossy, ridged, with folds radiating from the centre of the colony. White aerial mycelium appearing in small tufts at a late stage. This is easily observed on mannite lacking in nitrogen, and used for the study of nitrogen-fixing bacteria. Microscopically, aerial mycelium was found to be scant, with numerous dark staining granules in the hyphae. Small oval conidia fairly numerous, 1. x 0.6  $\mu$ . Filaments show a club-shaped appearance at the end.

*Hab.* California upland soil. Herbarium Nos. 45, 53 and 54.

STRAIN No. 3. Colony is of a dirty gray color, with an intensive ring formation. Scant white aerial mycelium develops when culture is 6 to 8 weeks old. Microscopically, aerial mycelium can hardly be differentiated; it shows a granular structure, with the formation of clubs at the end of the filaments. Some filaments are greatly enlarged. No conidia were observed.

*Hab.* Isolated once from garden soil 20 inches deep. Herbarium No. 36.

STRAIN No. 4. Colonies large, 5 to 8 mm. in diameter, at first gray, then becoming brown in color. Surface glossy, at first smooth, later becoming slightly wrinkled. Snow-white aerial mycelium is produced. No conidia and no clubs could be observed under the microscope. Aerial mycelium was found to be very dense.

*Hab.* California loam. Herbarium No. 22.

*Act. exfoliatus*, n. sp.

*Czapeck's Agar.* Colonies round, 2 to 3 mm. in diameter, of a Dresden-brown color (Rdg. xv-17'-k), with a wide sterile margin. Colony has a tendency to crack, and surface growth to exfoliate and peel off. The margin of the streak culture is peeled off, leaving medium free. Many cracks are found in centre of growth. White aerial mycelium is produced at an early date. A blue pigment is produced in the colony, not soluble in the substratum, but seen clearly through the aerial mycelium. Reverse of colony is brown to black. Microscopically, the aerial mycelium is found to be thick,  $1.5\mu$  in diameter. Conidia oval,  $1.2$  to  $1.8 \times 1$ . to  $1.5\mu$ . Odor weak.

*Gelatin.* Colony develops in the bottom of a liquefied pit, showing a dense yellow mycelium in the centre; edge of colony is hyaline. Radial mycelium extends into the unliquefied portion of the gelatin. White aerial mycelium is produced. Gelatin around the colony is slowly liquefied, with no color produced.

*Potato plug.* Growth continuous, thick, somewhat folded, at first colorless to gray, later becoming yellow. No aerial mycelium produced. Potato not affected.

*Czapeck's solution.* Many minute individual colonies all through medium and heavy pellicle on surface of liquid.

*Hab.* Isolated several times from the adobe and upland soils. Herbarium Nos. 20, 50 and 51.

*Act. diastaticus* (?), Krainsky.

*Czapeck's Agar.* Colonies 2 to 4 mm. in diameter, gray, later becoming colored pale yellow. Many rings are formed by growth of colony. Aerial mycelium drab-gray (Rdg. xlv-17""d), with small white tufts protruding in several places. Reverse of colony brown to black, with the deep brown mycelium penetrating deep into the substratum. This organism seems to answer Krainsky's description of the "diastaticus." However, no biochemical studies have been undertaken as yet to prove the identity of this organism. Microscopically, two kinds of aerial mycelium were found. The white mycelium is very dense, made up of straight, branching filaments. The brown aerial mycelium is made up of dense clusters of fine and narrow spirals. Oval conidia,  $1.1$  to  $1.5 \times 1$ . to  $1.2\mu$ . Odor weak.

*Gelatin.* Small colonies with white aerial mycelium produced at a late stage. Gelatin around the colony is rapidly liquefied, with no coloration.

*Potato plug.* Colonies small, 1 to 1.5 mm. in diameter, covering all the plug. Color of colonies white-gray with white aerial mycelium, which later becomes ash-gray. Each colony is pitted and raised 1 to 2 mm. above surface of plug. Potato darkened in two days.

*Czapeck's solution.* Flaky, colorless growth all through medium and on surface.

*Glucose solution.* Heavy gray ring of colonies on surface of liquid.

*Hab.* Isolated once from California sandy loam. Herbarium No. 13.

*Act. albus*, Krainsky.

This is a very common soil organism, isolated by Krainsky and reported by many investigators, as one of the few actinomyces found in the soil. The identification of this organism was based on Krainsky's description of the *Act. albus*.

*Czapeck's Agar.* Colonies 3 to 5 mm. in diameter, uniform in size, pale neutral-gray color (Rdg. liii-n. g.-d). White aerial mycelium produced early. Uniform growth on slant. Odor weak. Profuse spore formation. Conidia are both spherical and oval, 1.2 to 1.6 x 1.1 to 1.4 $\mu$ .

*Gelatin.* White aerial mycelium develops readily on the surface of the colony. Gelatin is liquefied rapidly with the production of a brown coloration.

*Potato plug.* Thin gray to brownish streak covered with white aerial mycelium, which becomes gray with age. Potato is not colored.

*Czapeck's solution.* Growth consists of small, white, individual colonies, 1 to 2 mm. in diameter, on glass of flask. None through medium and on bottom.

*Glucose solution.* Small growth consisting of a white ring on surface close to glass of flask.

*Hab.* Isolated several times from the adobe soil and garden soil at a depth of 8 inches. Herbarium Nos. 6, 30 and 63.

*Act. alboatrus*, n. sp.

*Czapeck's Agar.* Colonies colorless and fairly large, 5 to 8 mm. in diameter. Surface of colony at first smooth, later becoming ridged, with folds radiating from the centre. White aerial mycelium covers the whole colony at an early date, leaving a narrow glossy margin, which has a lichnoid appearance. Small drops of water are exuded upon the surface. Reverse of colony is at first light brown, becoming with age dark reddish-brown. Strong odor is present. Microscopically, aerial mycelium consists of dense, closely branched filaments. These break up into long fragments, which have nothing in common with what is ordinarily understood as conidia. No true conidia observed.

*Gelatin.* Gelatin is rapidly liquefied, with no coloration.

*Potato plug.* Growth forms a thick, continuous streak, much folded, glossy, and of a white color. Potato does not change in color. A rose-colored aerial mycelium appears only after 20 days at 30° C.

*Czapeck's solution.* Flaky growth on the bottom of the liquid, with few small colonies on the surface.

*Hab.* Isolated once from the adobe soil. Herbarium No. 52.

*Act. reticuli*, n. sp.

*Czapeck's Agar.* Colony colorless, 3 to 5 mm. in diameter, becoming covered soon with a thin white cottony growth. This white aerial mycelium is characteristic of the organism. It is very fine, forming a woven net over the whole surface of the colony, with holes of about 0.5 mm. Reverse of colony creamy, later becoming brownish. Aerial mycelium abundant, consisting of filaments having no branches or very short ones. Spherical conidia are abundant, 1. to 1.4 $\mu$ . Odor weak.

*Gelatin.* Rapid liquefaction takes place with the production of a soluble brown pigment only after 6 days.

*Potato plug.* Growth consists of brown, numerous colonies, 0.25 to 3 mm. in diameter, all over plug. Colonies are pitted, and covered with white aerial mycelium. Potato darkened.

*Hab.* Isolated from upland and adobe soils. Herbarium Nos. 43 and 95.

*Act. citreus*, Krainsky.

*Czapeck's Agar.* Colonies 3 to 5 mm. in diameter, with centre raised above substratum. Color of colony varying from olive-yellow (Rdg. xxx-23'') to citron-yellow (Rdg. xvi-23-b). Aerial mycelium of same color as colony, covering all colony without leaving any free margin. Reverse of colony deep colonial buff (Rdg. xxx-20'-b) to a deep yellow color, becoming with age yellow brown. Color of medium is unchanged. Odor weak. Microscopically, the substratum mycelium is found to consist of fine, branching filaments. Aerial mycelium consists of short, much branching, and tangled filaments. Conidia numerous, spherical to slightly oval, 1.2 to 1.8 x 1.2 to 1.5 $\mu$ . They do not stain evenly, showing a clear centre.

*Gelatin.* Colonies very dense, yellowish, with white aerial mycelium. Gelatin around the colony is rapidly liquefied with no discoloration.

*Potato plug.* Growth appears as a gray to yellow thin smear, with white aerial mycelium. Color of potato unchanged.

*Czapeck's solution.* Slight flaky growth on the bottom of the flask, medium remaining clear.

*Hab.* Isolated several times from the orchard soil at depths of 1 inch and of 20 inches, and from the garden soil at a depth of 20 inches. Herbarium Nos. 29, 82 and 87.

*Act. flavus*, Krainsky.

*Czapeck's Agar.* Colonies 3 to 5 mm. in diameter, olive-yellow (Rdg. xxx-23'') in color, showing a characteristic ring formation. Growth on streak has a tendency to form individual colonies. Aerial mycelium light drab (Rdg. xlv-17'''-C), with a wide sterile margin. Reverse of colony yellow to olive-yellow; no soluble pigment produced. The microscope

shows tangled aerial mycelium with little branching. The mycelium is swollen at intervals, forming the characteristic club shaped filaments up to  $3\mu$  in diameter. Spores oval and spherical,  $0.9$  to  $2 \times 0.9$  to  $1.1\mu$ .

*Gelatin.* Growth dense, light yellow in color, with a shiny surface; no aerial mycelium is produced. Liquefaction of the gelatin around the colony starts early, but does not advance very rapidly. A light brown pigment is produced.

*Potato plug.* A finely wrinkled continuous growth gray to brown in color is produced in two days. White aerial mycelium develops only after 8 to 10 days' incubation at  $30^{\circ}$  C. Potato colored brown.

*Czapeck's solution and Glucose solution.* Flaky growth on bottom of the flask, none through medium.

*Hab.* Isolated from upland and adobe soils. Herbarium Nos. 23 and 38.

*Act. parvus*, Krainsky.

*Czapeck's Agar.* Colony 1 to 3 mm. in diameter, of a honey-yellow color (Rdg. xxx-19"). Surface of colony smooth and glossy, long remaining without any aerial mycelium. Scanty gray-yellow aerial mycelium appearing only late. Reverse of colony brownish. Substratum mycelium fine, very dense, and tangled. Aerial mycelium consisting of thicker branching filaments. Conidia oval,  $1.2$  to  $1.8 \times 0.9$  to  $1.3\mu$ . No odor could be detected.

*Gelatin.* Colonies yellow, slowly developing on the gelatin. Liquefaction of the gelatin around the colony advances slowly, with no pigment production.

*Potato plug.* Growth continuous, folded, with a lichnoid margin, gray to brown in color. White aerial mycelium produced at an early stage. Potato colored black.

*Hab.* Isolated from garden soil at a depth of 12 inches, and Oregon soil. Herbarium No. 76.

*Act. griseus*, Krainsky.

*Czapeck's Agar.* Colony round, 3 to 6 mm. in diameter, growing rapidly with the formation of numerous rings. Color of colony olive-buff (Rdg. xl-21"-d). Aerial mycelium appears at an early stage; it is a thick powdery mass of a water-green color (Rdg. xli-25"-d). The color of the aerial mycelium is somewhat lighter than that described by Krainsky. Odor present, but weak. Microscopically, the aerial mycelium is found to consist of long filaments, with very little branching. These fragment readily into rod-shaped conidia,  $1.$  to  $1.5 \times 0.8\mu$ . The conidia occur often in chains; they do not stain readily in the centre; so that they produce a beaded effect.

*Gelatin.* Greenish-yellow colonies with a prominent substratum

growth. Aerial mycelium produced of a white-gray color. Liquefaction of the gelatin takes place rapidly with no pigment production.

*Potato plug.* Growth yellow, continuous, with the formation of individual colonies separated from the streak. Aerial mycelium at first white-gray, then changing into the characteristic water-green color. Potato is colored brown.

*Czapeck's solution.* Flaky growth on the bottom and throughout the medium.

*Glucose solution.* Heavy growth consisting of round colonies (1 to 3 mm. in diameter) floating on the surface and forming a ring in contact with the glass. A powdery white aerial mycelium soon covers the surface of the colonies. Some growth is found on the bottom of the liquid.

*Hab.* Isolated from California adobe soil. Herbarium Nos. 33 and 34.

*Act. albo-flavus*, n. sp.

*Czapeck's Agar.* Colonies small (2 to 4 mm.), round, colorless and glossy, at first, later becoming yellowish in color. Aerial mycelium forming a white powdery mass, with a yellow tinge developing later. Reverse of colony is yellowish, but medium is not colored. Under the microscope the aerial mycelium was found to have a tendency to produce special structures, consisting of a mass of hyphae massed together into a rope, and from this rope fine filaments coming out in the shape of side branches. The structure looks like the root of a tree and fine rootlets coming out on the side. This special structure persisted even in stained preparations. No conidia were observed. No spirals produced. Weak odor present.

*Gelatin.* Rapid liquefaction of the gelatin with no pigment production. The colonies are floating in the liquefied portion. No aerial mycelium produced.

*Potato plug.* Gray, continuous, thin growth. White aerial mycelium produced in 2 days at 30° C. Color of potato not changed.

*Czapeck's solution.* Few small colonies on the glass, none through medium or on bottom.

*Glucose solution.* White cylindrical colonies 3 x 1 mm., very uniform in size, growing together in a mass on the surface of the liquid. White aerial mycelium is produced at an early stage. The type of colony is very characteristic of this species, because it has not been observed in any other culture.

*Hab.* Isolated once from orchard soil at a depth of 20 inches. Herbarium No. 10.

*Act. Verne*, n. sp.

*Czapeck's Agar.* Colony 3 to 6 mm. in diameter, much folded, of an Isabella color (Rdg. xxx-19-i), with a wet surface. Margin of colony lichnoid. Aerial mycelium is little differentiated from the surface of the colony, and no such could be demonstrated. An elm-green (Rdg. xvii-27-

k m) soluble pigment is produced, which diffuses rapidly all through the medium. Weak odor present. Microscopically, substratum mycelium found to be very fine, radial. Surface mycelium thicker and much branched. No segmentation or conidia could be demonstrated. Raised portion of old growth consists of a hard yellowish amorphous crust breaking into irregular fragments; there is no true aerial mycelium.

*Gelatin.* Globular colonies grow below the surface of the liquefied portion of the gelatin, varying in size from microscopic to 1.5 mm. No aerial mycelium is produced. Reverse of colonies is dark, due to the green pigment discoloring the medium. Gelatin is rapidly liquefied with no brown coloration.

*Potato plug.* Growth at first yellowish-gray, with a tendency to form individual colonies. Later it becomes thick and much folded. Scant white aerial mycelium is produced only on the tip of the growth. Color of potato is unchanged.

*Czapeck's solution.* Small colonies formed on the bottom of the liquid.

*Glucose solution.* Slight flaky growth on the bottom.

*Hab.* Isolated once from the upland soil. Herbarium No. 42.

*Act. albosporus*, Krainsky.

*Czapeck's Agar.* Colony Acajou red (Rdg. xiii-l'-1), with an early production of white aerial mycelium. Reverse of colony orange-red. Growth on streak continuous, without any tendency to form individual colonies. Answers closely Krainsky's description. Substratum mycelium fine, radial, red colored. Aerial mycelium coarser, white, much branched. No spirals produced. Conidia very distinct, formed readily; spherical and oval shaped 1. to 1.8 x 0.8 to 1.2 $\mu$ , often occurring in chains.

*Gelatin.* Colonies at first yellow in centre and hyaline at the margin; later they become red colored, remaining hyaline at the margin. Gray mycelium is produced in the centre of the colony. Gelatin is rapidly liquefied, with no pigment production.

*Potato plug.* Growth very slight, translucent, gray, becoming orange colored, with white aerial mycelium.

*Czapeck's solution.* Small flakes all through the medium and on the surface with the production of a soluble rose pigment.

*Glucose solution.* A pinkish ring is formed at the surface in contact with the glass; fair growth downward in the medium.

*Hab.* Isolated once from the upland soil. Herbarium No. 26.

*Act. Bobili*, n. sp.

*Czapeck's Agar.* Colonies small, 1.5 to 2.5 mm., folded, with a much cut lichnoid margin. Color at first coral red (Rdg. xiii-5'), becoming with age Acajou to Pompeian red (Rdg. xiii-l'-3'i), with a light colored margin. Reverse is light red. No true aerial mycelium is produced.



Odor strong, mouldy. Microscopically, the surface mycelium was found to be very fine and dense. No true aerial mycelium or spores could be demonstrated. Old surface growth consisting of a mass of degenerated mycelium.

*Gelatin.* Colonies dense, some having slight aerial growth. Each colony found at the bottom of a liquefied pit. Gelatin rapidly liquefied, at first colorless, then becoming colored brown. Under the microscope peculiar hyaline aerial formations were observed in the form of wedge, up to  $10\mu$  wide at the base, and tapering to a point. When these were smashed under the cover slip, nothing more could be found.

*Potato plug.* Growth gray to red, spreading all over plug. Surface of growth dry and much folded. Scant white aerial mycelium formed late at the tip of plug. Potato becoming brown with age.

*Czapeck's solution.* Colonies, 2 to 3 mm. in diameter, found all through liquid and on the bottom. Colonies are colorless at first, later the centre of the colonies becomes orange.

*Glucose solution.* Flaky growth on bottom, none on surface.

*Hab.* Isolated from adobe and garden soils. Herbarium Nos. 15 and 37.

*Act. Californicus*, n. sp.

*Czapeck's Agar.* Colonies small, 1 to 2 mm., round, vinaceous colored (Rdg. xxvii-1"-d), growing deep into the substratum with almost no surface growth. The growth of the mycelium into the substratum can easily be followed by the red growth penetrating, 1 to 2 cm. deep, into the medium. No soluble pigment is produced, the color is found in the medium only where the mycelium has penetrated. Light neutral gray (Rdg. liii-n. g.-C) aerial mycelium covers surface in the form of a dry, powdery, thin layer. Microscopically, an abundant formation of open spirals could be found,  $3.5$  to  $6\mu$  in diameter. These break up easily into perfectly spherical conidia, which are very uniform and quite numerous,  $1.2$  to  $1.5\mu$  in diameter, often occurring in long chains. No odor could be detected.

*Gelatin.* Dense colorless colonies are abundant, with a gray-white aerial mycelium. Gelatin around the colony is slowly liquefied, with no pigment production.

*Potato plug.* Growth at first yellow to red, glossy, and shiny, with age becoming reddish-brown. No aerial mycelium produced. Potato not changed in color.

*Czapeck's solution.* Thin flaky growth all through medium and on surface.

*Glucose solution.* Pinkish ring formed at the surface, close to the glass. Scant flaky growth through medium.

*Hab.* Isolated once from the California sandy loam. Herbarium No. 3.

*Act. Lipmannii*, n. sp.

*Czapeck's Agar.* Colony 3 to 5 mm. in diameter, at first colorless, later becoming light brown. Centre of colony is elevated, giving it a conical appearance. There is an abundant production of aerial mycelium, which changes from neutral-gray (Rdg. liii. n. g.) to gray (Rdg. liii-6), with a white margin and white tufts all over surface. A ring formation takes place, alternating gray and white zones. Surface is smooth or slightly ridged. Minute drops of water exude upon the surface, giving the growth a silvery appearance. Reverse of colony is first light brown, then changes to almost black. No soluble pigment is produced. Under the microscope the aerial mycelium is found to be much branching and fragmenting readily into spherical or oval conidia. The conidia are 1. to 1.5 x 0.8 to 1.1 $\mu$ , often occurring in chains. Odor weak.

*Gelatin.* Growth colorless, with white-gray aerial mycelium. Gelatin is rapidly liquefied with no pigment production. One strain of this organism (4) produced a cerro-green (Rdg. v-27-m) growth with white aerial mycelium.

*Potato plug.* Growth on the potato varies slightly with the different strains of the organism. It is usually a folded white to brownish smear, turning gray to gray-green. Aerial mycelium is white to ashy-gray. Color of the potato remains unchanged. The smear of one strain (12) is sulphur-yellow, of another (4) olive-green; otherwise the characters are alike.

*Czapeck's solution.* Flaky growth on bottom and surface, small white colonies all through the liquid.

*Glucose solution.* Grayish-white ring formed at the surface, close to glass.

*Hab.* This very common soil organism has been isolated by the writers many times from different soils, from the adobe, California sandy loam, and garden soil at a depth of 30 inches. Herbarium Nos. 4, 5, 7, 12, 58 and 62.

*Act. Rutgersensis*, n. sp.

*Czapeck's Agar.* Colony 3 to 8 mm. in diameter, slightly raised in the centre. Substratum mycelium penetrates deep into the medium. Surface colorless with an irregular margin. Aerial mycelium is produced at an early stage; it is at first gray, later becoming pale gull-gray (Rdg. liii-c. g.). Zonation takes place in the formation of alternate gray and white rings. Reverse of colony changes from white to brownish. Microscopically, the aerial mycelium is found to consist of long, branching filaments, loose at the margin, but dense in the centre. There is an abundant spiral production of the close and open type. Conidia spherical and oval, 1. to 1.2 $\mu$  in diameter, having a tendency to bi-polar staining. A strong odor is produced.

*Gelatin.* Rapid liquefaction of the gelatin takes place with no pigment production. No aerial mycelium formed.

*Potato plug.* Growth varies from a gray to a dark solid streak with scant formation of white aerial mycelium. Color of potato not changed.

*Hab.* This form, another common soil organism, has been isolated repeatedly by the writers from the local soils, from garden soil at depths of 1, 20 and 30 inches, orchard soil at 4 and 20 inches, timothy soil at 4 inches. Herbarium Nos. 67, 75, 79, 80, 83, 86 and 91.

*Act. aureus*, n. sp.

*Czapeck's Agar.* Colony 4 to 5 mm. in diameter, and translucent in color when 7 days old. Surface smooth. Aerial mycelium appears at an early date, at first mouse-gray (Rdg. li-15'''), then changing into a cinnamon-drab color (Rdg. xlvii-13'''). Ring formation takes place by the alternation of white and drab colored zones. Reverse at first white, changing to brown and almost dark brown. No soluble pigment produced. A characteristic exudation of dirty gray drops of water takes place in the centre of the colony, forming a small ring. Weak odor present. Aerial mycelium is characterized by the formation of numerous long spirals 17 to 20 $\mu$  long, and 4 to 5 $\mu$  in diameter. Spherical and oval conidia formed abundantly, 1. to 1.5 x 1. to 1.2 $\mu$ . They stain readily, sometimes in a bi-polar manner.

*Gelatin.* Liquefaction starts rapidly in 3 to 4 days at 15° to 17° C., with no pigment production; then it becomes slower, with the production of a deep brown pigment in the unliquefied portion. White aerial mycelium is produced.

*Potato plug.* Growth continuous, folded, raised above the potato, of a gray to brown color. White to gray aerial mycelium appears early. Color of potato becomes black.

*Hab.* This forms also a common and numerous group of soil organisms. It has been isolated repeatedly from the local soils; garden soil at a depth of 1 inch, orchard soil at 12 and 30 inches, timothy soil at 4 inches, and Oregon soil. Herbarium Nos. 66, 68, 70, 71, 84 and 89.

*Act. Halstedii*, n. sp.

*Czapeck's Agar.* Colony gray, translucent, 4 to 8 mm. in diameter when 7 days old. Centre of colony is dark, with a large hyaline margin. Surface smooth. Aerial mycelium appears at an early date; it is at first white, then gull-gray (Rdg. liii-c.g.). Reverse of colony is colorless, turning dark in the centre. Medium not discolored. Odor fairly strong. Microscopically, the gray aerial mycelium was found to consist of long, slender, and spreading filaments. Close spirals, 7 to 10 $\mu$  in diameter, are borne as branches of the filaments. Conidia are oval to rod-shaped, 1.2 to 1.8 x 1. to 1.2 $\mu$ , often occurring in chains; they show only polar staining.

*Gelatin.* Rapid liquefaction with the production of a brown pigment in the unliquefied portion.

*Potato plug.* Growth solid, folded, greatly raised above the potato, gray to brown in color. White aerial mycelium covers only tip of growth. Color of potato is changed to black.

*Czapeck's solution.* Growth consists of 1 to 2 mm. colonies on side of vessel and bottom.

*Hab.* This is a common subsoil organism, isolated repeatedly from the deeper soil layers, but not from the surface soil. Garden soil at depths of 12, 20 and 30 inches; orchard soil at 12, 20 and 30 inches. Herbarium Nos. 33, 56, 72, 77, 85.

*Act. Fradii*, n. sp.

*Czapeck's Agar.* Colony 2 to 4 mm. in diameter, colorless, thin, with a smooth surface. Aerial mycelium is produced early; it is a thick cottony mass of a sea-shell pink color (Rdg. xiv-11'-f), with white tufts of mycelium in many places. Reverse colorless. No soluble pigment produced. Odor weak. Aerial mycelium consists of thick, long, unbranched filaments, which become branched only when old. Conidia numerous, rod-shaped, .75 to 1.25 x 0.5 $\mu$ , of a sea-shell pink color.

*Gelatin.* Rapid liquefaction with no color production. White aerial mycelium is produced early.

*Potato plug.* Growth glossy, thick, of a zinc-orange color (Rdg. xv-13'). White to rose aerial mycelium. Color of plug not changed.

*Czapeck's solution.* Numerous minute colonies all through medium and on surface.

*Hab.* Isolated once from the adobe soil. Herbarium No. 55.

*Act. roseus*, Krainsky.

*Czapeck's Agar.* Colony 2 to 3 mm. in diameter, of a pale brownish vinaceous color (Rdg. xxxix-5'''-f). Growth of colony is limited. Aerial mycelium is of the same color as the colony, and is produced at an early date. Medium uncolored. No odor or odor very weak. The general characters of the organism coincide closely with those given by Krainsky. Microscopically, the species is characterized by the formation of numerous close spirals. Conidia formation takes place early; they are abundant and oval in shape, 1.5 to 2 x 1. to 1.2 $\mu$ .

*Gelatin.* Slow liquefaction of the gelatin with the production of a deep brown pigment which spreads rapidly through the unliquefied portion of the gelatin.

*Potato plug.* Gray-yellow continuous streak on the plug. White aerial mycelium covers only tip of growth. Color of potato turns brown.

*Czapeck's solution.* Heavy, flaky mass all through liquid and on surface.

**Glucose solution.** Pink ring on the surface close to the glass. Some growth on the bottom.

**Hab.** Isolated from garden soil at depths of 8 and 12 inches. Herbarium Nos. 27 and 73.

*Act. lavendulae*, n. sp.

**Czapeck's Agar.** Colony 3 to 4 mm. in diameter, colorless, growing deeply into the medium in the form of long, colorless strands, with very little surface growth. Aerial mycelium appearing early in centre of colony; it is deep vinaceous lavender (Rdg. xlv-65'''-d), with a large sterile margin. A strong odor is present. Microscopically, close spirals were found, 5 to  $8\mu$  in diameter. Conidia abundant, oval, 1.6 to 2. x 1. to  $1.2\mu$ .

**Gelatin.** Slow liquefaction with the production of a brown pigment only after 6 days.

**Potato plug.** Golden brown, wide, thin, continuous growth. Color of potato black.

**Hab.** Isolated once from orchard soil at a depth of 4 inches. Herbarium No. 69.

*Act. purpurogenus*, n. sp.

**Czapeck's Agar.** Colony gray-translucent, 3 to 6 mm. in diameter, radially much wrinkled. Centre of colony is elevated, and colony itself becomes lichnoid in appearance. Centre is covered by white to purplish aerial mycelium, shading into dark grayish lavender (Rdg. xliii-57'''-C). Reverse of colony brownish to dark brown. No soluble pigment produced. Odor weak. Aerial mycelium is found under the microscope to be dense and twisted. Conidia oval, 1. to  $1.5 \times 0.8$  to  $1\mu$ .

**Gelatin.** Slow liquefaction with purplish coloration. White aerial mycelium is produced at an early date.

**Potato plug.** Gray-dark, much folded, continuous growth, with a pearly lustre. It looks as if many small individual colonies were massed together to form the streak. White aerial mycelium appearing late. Color of potato turned black.

**Czapeck's solution.** Large, thin, radiating colonies, rarely through medium and on surface.

**Hab.** Isolated repeatedly from the garden soil at depths of 20 and 30 inches, and orchard soil at 20 inches. Herbarium Nos. 59, 65 and 90.

Besides the classified organisms, several more cultures of unidentified species are at hand. Most of these develop very slowly, and not enough data could be collected for any grouping. They are kept for further study.

TABLE IV.  
TABULAR STATEMENT OF SALIENT FEATURES OF ACTINOMYCES.

Name of Organism	Growth on Czapek's Agar				Gelatin (15% in dist. H <sub>2</sub> O)			Liquid Czapek	Dextrose Broth 1%	Potato Plug			Odor
	Color of Colony	Aerial Mycelium	Reverse	Medium Colored	Liquefaction	Color of Medium	Aerial Mycelium			Color of Colony	Aerial Mycelium	Plug Colored	
<i>Act. violaceus-ruber</i> .....	red and blue	gray	red and blue	blue	slow	none	present white	flaky	surface	gray	white	blue	weak
<i>Act. violaceus-Caesi</i> .....	gray	white	blue	plum-purple	rapid	none	none	flaky	bottom flaky	yellowish	scant, white	not colored	weak
<i>Act. violaceus-niger</i> .....	gray-black	white	black	black	rapid	none	none	colonies	.....	brown	white	black	medium
<i>Act. erithro-chromogenus</i> ....	brown	white	brown	brown	slow	brown	present white	colonies	scant growth	yellow-gray	none	black	strong
<i>Act. diastato-chromogenus</i> ....	gray	white-gray	brown	brown	rapid	brown	none	flaky	flaky	gray	white	black	medium
<i>Act. purpeo-chromogenus</i> ....	brown	purple	purple	brown	slow	brown	none	flaky	.....	orange	none	not colored	none
<i>Act. virido-chromogenus</i> ....	green-brown	black	black	black	rapid	brown	none	.....	.....	brown green	white	black	medium
<i>Act. chromogenus (group)</i> ....	brown	white	black	brown	rapid	brown	none	.....	.....	brown	white	black	medium
<i>Act. exfoliatus</i> .....	brown	white	black	not colored	slow	none	present	colonies	.....	gray-yellow	none	not colored	very weak
<i>Act. diastaticus</i> .....	gray	drab	dark	not colored	rapid	none	present	flaky	ring	gray-white	white	dark	weak
<i>Act. albus</i> .....	gray	gray white	gray	not colored	rapid	brown	present	colonies	ring	brown	white-gray	not colored	weak
<i>Act. albostrus</i> .....	colorless	gray white	brown	not colored	rapid	none	none	flaky & colonies	.....	white	rosy	colored	strong
<i>Act. reticuli</i> .....	colorless	white	creamy	not colored	rapid	brown	none	.....	.....	brown	white	dark	weak
<i>Act. citreus</i> .....	olive	gray white and light	gray	not colored	rapid	none	white	flaky	little growth	gray	white & yellow	not colored	weak
<i>Act. flavus</i> .....	yellow	drab	yellow to olive-yel.	not colored	slow	brown	none	flaky	flaky	black	white	not colored	none

TABLE IV—(Continued).  
TABULAR STATEMENT OF SALIENT FEATURES OF ACTINOMYCES.

Name of Organism	Growth on Czapeck's Agar				Gelatin (15% in dist. H <sub>2</sub> O)			Liquid Czapeck	Dextrose Broth 1%	Potato Plug			Odor
	Color of Colony	Aerial Mycelium	Reverse	Medium Colored	Liquefaction	Color of Medium	Aerial Mycelium			Color of Colony	Aerial Mycelium	Plug Colored	
<i>Act. parvus</i> .....	yellow	yellow	brown	not colored	slow	none	none	.....	.....	gray	white	black	none
<i>Act. griseus</i> .....	olive buff	water-green white	brownish	not colored	rapid	none	present	flaky	colonies	yellow & gray	white-gray	brownish	weak
<i>Act. albiflavus</i> .....	yellowish	white	yellowish	not colored	rapid	none	none	colonies	cylindrical colonies	gray	white	not colored	weak
<i>Act. Verne</i> .....	Isabella color	yellow-brown	dark	green	rapid	none	none	colonies	flaky	yellow gray	scant, white	not colored	weak
<i>Act. albosporus</i> .....	Acajou red	white	red	not colored	rapid	none	present	flaky	flaky	orange	white	not colored	none
<i>Act. Bobili</i> .....	red	none	red	not colored	rapid	brown (late)	none	colonies	flaky	red	none	not colored	strong
<i>Act. Californicus</i> .....	red	gray	red	colored	slow	none	present	flaky	flaky	red	none	not colored	none
<i>Act. Lipmanii</i> .....	colorless	gray	dark	colored	rapid	none	white-gray	flaky & colonies	ring	.....	.....	colored	weak
<i>Act. Rutgersensis</i> .....	colorless	gray	white	not colored	rapid	none	none	.....	.....	gray	white	not colored	strong
<i>Act. Halsteadii</i> .....	gray to dark	white	colorless	colored	rapid	brown	present	.....	.....	brown	white	black	strong
<i>Act. aureus</i> .....	colorless	drab	white to brownish	not colored	rapid	brown	present	.....	.....	.....	white	black	weak
<i>Act. Fradii</i> .....	colorless	sea-shell pink	creamy	not colored	rapid	none	white	colonies	.....	orange	rose	not colored	weak
<i>Act. roseus</i> .....	rose	rose	light	colored	rapid	brown	present	flaky	flaky	gray	white	brown	none
<i>Act. lavendulae</i> .....	colorless	lavender	rosy	colored	slow	brown	none	.....	.....	brown	none	black	strong
<i>Act. purpurigenus</i> .....	gray	gray to lavender	brown	colored brownish	slow	purplish	white	.....	.....	dark	white	black	weak

## KEY TO THE IDENTIFICATION OF THE ACTINOMYCES.

## A. Gelatin liquefied rapidly, with no pigment produced in the unliquefied portion:

## I. Spirals produced in the aerial mycelium:

1. No pigment produced in the substratum, *Act. Rutgersensis*
2. Pigment produced in the substratum:
  - (a) Pigment dark blue, *Act. violaceus Caeseri.*
  - (b) Pigment brown, *Act. diastaticus.*

## II. No spirals produced in the aerial mycelium:

1. No pigment produced in the substratum:
  - (a) Colony orange-red, aerial mycelium white, *Act. albosporus.*
  - (b) Colony rose-colored, aerial mycelium rosy, *Act. Fradii.*
  - (c) Colony a mixture of white and yellow:
    - (c') No conidia observed, *Act. albo-flavus.*
    - (c'') Conidia present in abundance:
      - x Conidia rod-shaped, colony powdery, gray-yellow. *Act. griseus.*
      - y Conidia spherical and oval, colony compact, citron-yellow. *Act. citreus.*
  - (d) Colony at first colorless, then becoming brown, to almost black:
    - (d') Aerial mycelium white, no conidia observed, *Act. albotratus.*
    - (d'') Aerial mycelium dark gray; conidia abundant, oval, *Act. Lipmanii.*
2. Pigment produced in substratum:
  - (a) Color of substratum green, *Act. Verne.*
  - (b) Color of substratum dark blue, *Act. violaceus-niger.*

## B. Gelatin liquefied rapidly with the production of a brown pigment in the unliquefied portion:

## I. Spirals produced in the aerial mycelium:

1. Colony rose-colored, with rosy aerial mycelium, *Act. roseus.*
2. Colony colorless, with golden brown aerial mycelium, *Act. aureus.*
3. Colony slightly brown, with white aerial mycelium, *Act. Halstedii.*

## II. Spirals not produced in the aerial mycelium:

1. No pigment produced on the agar substratum:
  - (a) Colony red to red-orange, with no aerial mycelium, *Act. Bobili.*
  - (b) Colony white, with white aerial mycelium:
    - (b') Aerial mycelium thin, rare, net-like, *Act. reticul.*
    - (b'') Aerial mycelium thick, white to gray, *Act. albus.*
2. Brown pigment produced in the agar:
  - (a) White aerial mycelium produced early and abundant, *Act. diastato-chromogenus.*
  - (b) White aerial mycelium not produced at all, or very late, *Act. chromogenus group.*
  - (c) Surface of colony green, white aerial mycelium produced early, *Act. virido-chromogenus.*



## C. Gelatin slowly liquefied, with no pigment production:

## I. Spirals produced in the aerial mycelium:

1. Production of soluble red and blue pigments, *Act. violaceus-ruber*.
2. No soluble pigment produced; red mycelium grows deep into the substratum, *Act. Californicus*.

## II. No spirals produced in the aerial mycelium:

1. No pigment produced in the substratum, colony yellow. *Act. parvus*.
2. Brown pigment produced, colony tends to crack, *Act. exfoliatus*.

## D. Gelatin slowly liquefied, with the production of a brown pigment:

I. Spirals produced: aerial mycelium lavender color, *Act. lavendulae*.

## II. No spirals produced in the aerial mycelium:

1. Colony yellow, aerial mycelium gray, *Act. flavus*.
2. Colony colorless, aerial mycelium white-purplish, *Act. purpureogenus*.
3. Colony black, lichnoid, aerial mycelium scant, *Act. erithrochromogenus*.
4. Colony purple, no aerial mycelium, *Act. purpeochromogenus*.

## PHYSIOLOGY OF THE ACTINOMYCES.

Investigators find that most of these organisms grow best at 30° C. The minimum lies near 15° C. and the maximum is about 50° C.

The cellulose destroying power of the actinomyces has been studied by Krainsky (13), Scales (22), and several others.

As to their ability to liberate ammonia, Lutman and Cunningham (15) by inoculating nutrient broth with *Act. chromogenus* found 10 to 20 mg. of  $\text{NH}_3$  in 50 c.c. of culture for from 14 to 30 days. Since liberation of ammonia from organic compounds is one of the most important factors in the study of organisms from the soil fertility standpoint, a series of experiments were started for the purpose of demonstrating the part played by the actinomyces in this process.

One hundred grams of soil and 2.42 gm. of cottonseed meal containing 155 mg. of nitrogen were placed in Erlenmeyer flasks and well mixed. Twenty cubic centimeters of water (equal to 70 per cent saturation for the soil used) were added. Two sets of flasks received 10 c.c. each and another two 40 c.c. each. Flasks were plugged and sterilized in the autoclave, 15 minutes at 15 pounds pressure. Cultures of the different organisms grown on Czapeck's solution were used for inoculation, 1 c.c. being added. Duplicates were used throughout. After 7, 14 and 30 days, respectively, the ammonia of the various sets was distilled off with  $\text{MgO}$  in the usual way. Table IV shows the results of the investigation.

One can readily see from Table V that the actinomyces do not play any appreciable rôle in the soil as ammonifiers, as judged by ammonification in soil sterilized by steam under pressure. Only six organisms gave more than 1 mg. of nitrogen as  $\text{NH}_3$ , all the rest accumulating less than that amount in 14 days. When the incubation period was extended to 30 days somewhat larger quantities of  $\text{NH}_3$  were found to have been

TABLE V.  
AMMONIA ACCUMULATION BY ACTINOMYCES IN THE SOIL.

Name of Organism	Period of Incubation	Moisture Content	Mg. of N. as Ammonia	Mg. of N. Average	Mg. of N. Minus check
Act. violaceus-ruber . . . . .	14 days	20%	4.06 4.36	4.21	0.51
Act. violaceus-Caeseri . . . . .	14 days	20%	3.64 4.10	3.87	0.17
Act. roseus . . . . .	14 days	20%	3.52 3.06	3.29	-0.41
Act. erithrochromogenus . . . . .	14 days	20%	4.06 4.67	4.37	0.67
Act. griseus . . . . .	14 days	20%	4.71 4.10	4.41	0.71
Act. griseus . . . . .	14 days	10%	3.33 3.46	3.40	-0.30
Act. griseus . . . . .	14 days	40%	7.01 7.16	7.09	3.39
Act. griseus . . . . .	30 days	20%	9.18 9.84	9.51	5.81
Act. albus . . . . .	7 days	20%	4.56 4.12	4.34	0.74
Act. albus . . . . .	14 days	20%	4.24 4.88	4.56	0.86
Act. albus . . . . .	14 days	10%	3.16 3.94	3.55	-0.15
Act. albus . . . . .	14 days	40%	7.01 7.63	7.32	3.62
Act. albus . . . . .	30 days	20%	19.2 19.8	19.5	15.8
Act. citreus . . . . .	14 days	20%	3.80 3.80	3.80	0.10
Act. flavus . . . . .	14 days	20%	3.75 4.06	3.91	0.21
Act. albosporeus . . . . .	14 days	20%	4.56 4.25	4.41	0.71
Act. albo-flavus . . . . .	14 days	20%	3.75 3.75	3.75	0.05
Act. Lipmanii . . . . .	14 days	20%	4.36 4.88	4.62	0.92
Act. Chromogenus (No. 22) . . . . .	14 days	20%	4.51 3.75	4.13	0.43

accumulated. When the different moisture contents employed are compared it is found that the highest moisture content gave the highest ammonia accumulation.

It has been found that the actinomyces readily assimilate  $\text{NO}_2$ ,  $\text{NO}_3$ ,  $\text{NH}_3$ , and organic compounds of nitrogen. The characteristic point is that they reduce nitrate to nitrite, but not to free nitrogen or ammonia.

Not all actinomyces produce an odor, but as all representatives of the *Act. chromogenus* group seem to have an odor, it was formerly believed that this property was characteristic of the whole genus. The odor, when present, varies from very strong to very weak. The stronger odors are suggestive of a musty straw stack, and some of the milder ones of the smell of soil.

Heinze (11) suggested that the actinomyces probably play an important part in the formation of humus out of the organic matter of the soil, even when the soil has an acid reaction. Fousek (18) found that "streptothrix" do not nitrify; on the contrary, they reduce nitrates to nitrites, but without loss of free nitrogen. In view of the ready assimilation of nitrates, ammonia compounds, urea, and uric acid, by these organisms, it seems possible that they may help to "fix" nitrogenous fertilizers in the soil and prevent their being lost by leaching or denitrification. He (Fousek) states that the "streptothrix" have a favorable influence upon plant growth, because through their rapid decomposition of the organic matter, plant nutrients are set free and made available for the higher plants.

The knowledge that the actinomyces are strong cellulose decomposers and weak producers of ammonia leads one to think that the probable rôle of the organism in the fertility of the soil lies in the formation of humus. Organic matter when applied to the soil has to undergo a series of processes before it can be utilized by the higher plants. Among the most important of these is the decomposition of cellulose, and subsequent formation of humus. In this process the actinomyces probably play an important, if not a dominant part, together with the cellulose decomposing bacteria and fungi.

In arid soils where cellulose destruction has been found to be extremely rapid, we should, therefore, expect to find actinomyces in abundance. That this might be the case is indicated by the figures in Table III, representing the soils from southern California.

#### ACKNOWLEDGEMENT.

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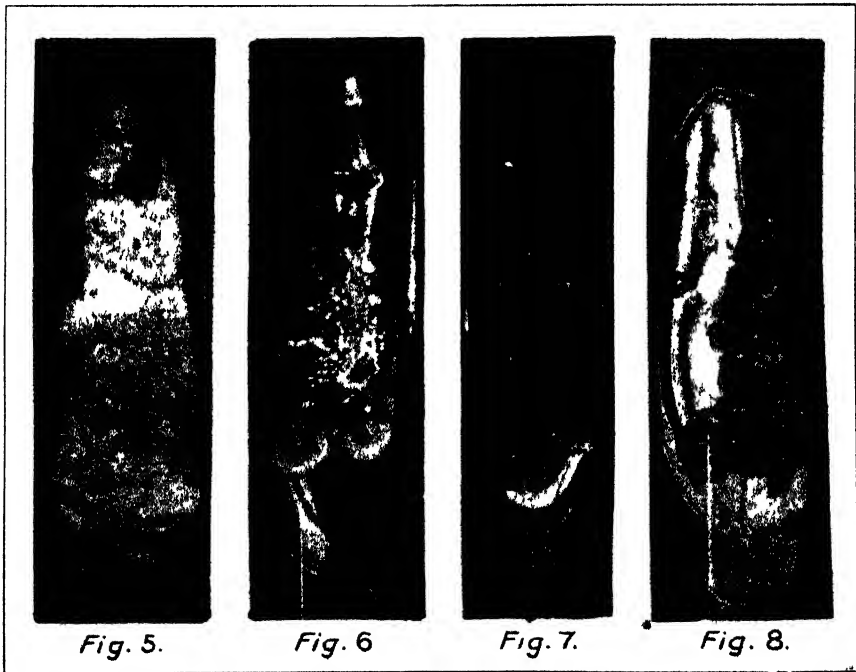
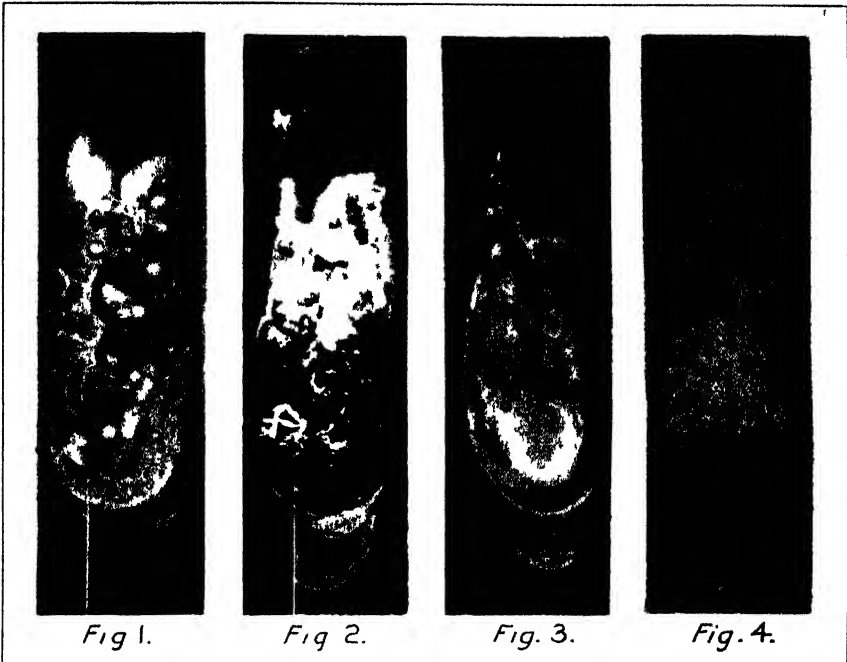
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# PLATE I.

- Fig. 1—Act. violaceus-ruber.
- Fig. 2—Act. violaceus-Caeseri.
- Fig. 3—Act. chromogenus, strain 22.
- Fig. 4—Act. virido-chromogenus.
- Fig. 5—Act. diastato-chromogenus.
- Fig. 6—Act. erithro-chromogenus.
- Fig. 7—Act. purpeo-chromogenus.
- Fig. 8—Act. chromogenus, strain 40.





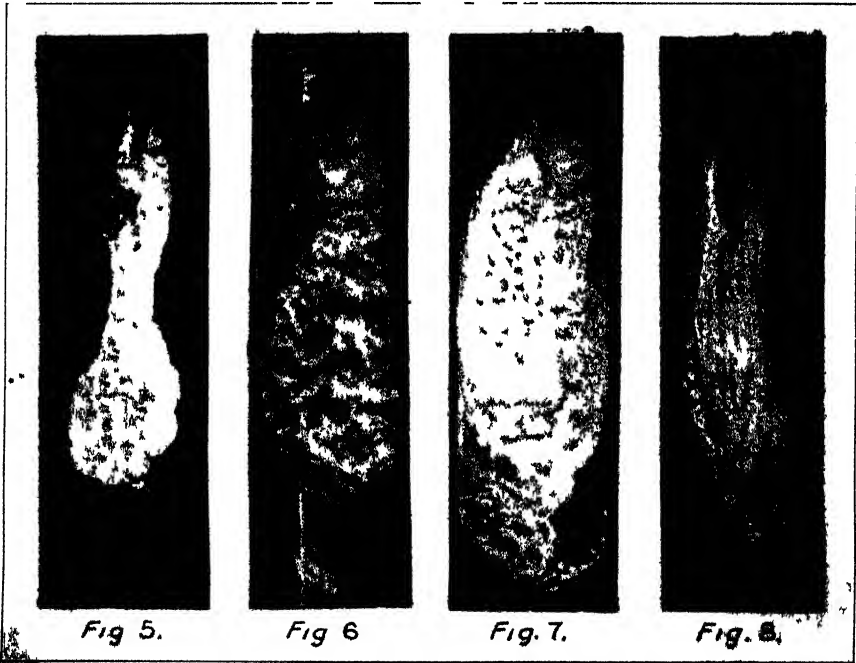
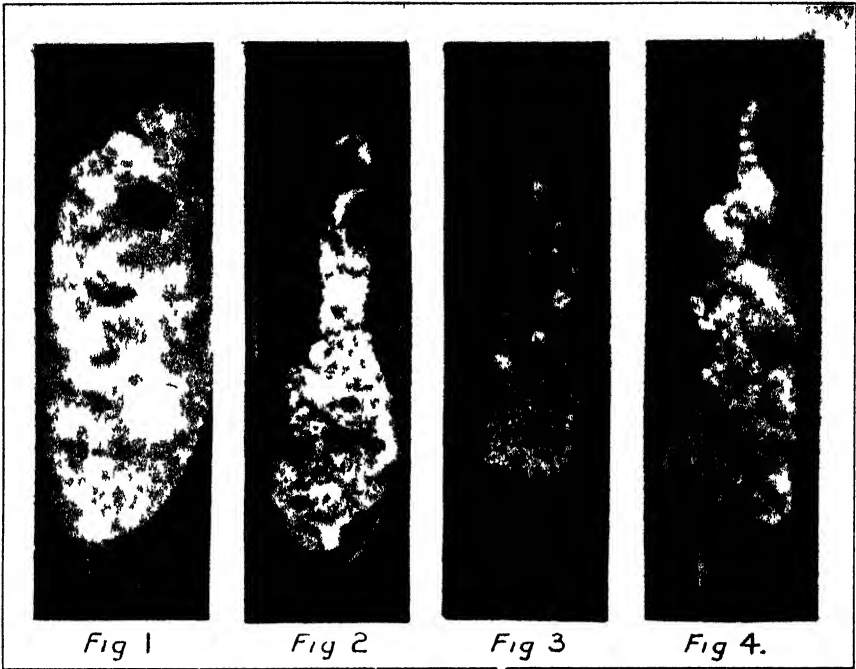
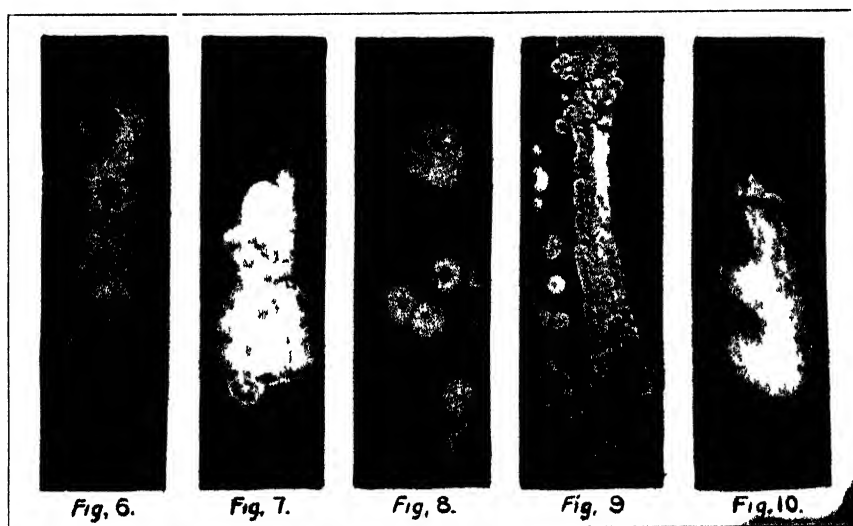
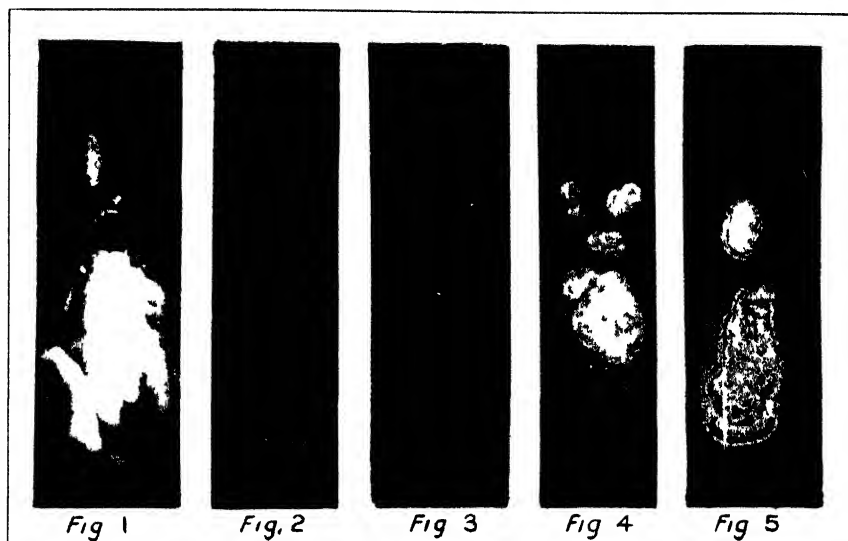


PLATE II.

- Fig. 1—Act. albus.  
Fig. 2—Act. albotratus.  
Fig. 3—Act. reticuli.  
Fig. 4—Act. alboflavus.  
Fig. 5—Act. albosporeus.  
Fig. 6—Act. Verne.  
Fig. 7—Act. griseus.  
Fig. 8—Act. Californicus.

### PLATE III.

- Fig. 1—*Act. citreus*.
- Fig. 2—*Act. Bobili*.
- Fig. 3—*Act. purpurogenus*.
- Fig. 4—*Act. Lipmanii*.
- Fig. 5—*Act. diastaticus*.
- Fig. 6—*Act. Fradii*.
- Fig. 7—*Act. exfoliatus*.
- Fig. 8—*Act. flavus*.
- Fig. 9—*Act. Rutgersensis*.
- Fig. 10—*Act. roseus*.





## STUDIES ON SOIL PROTOZOA.\*

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The question of the activities of protozoa in the soil raised by Russell and his associates has turned the attention of the workers in soil microbiology to this group of microorganisms as a possible factor in soil fertility. The literature dealing with soil protozoa is given in several of the latest investigations, and the writer will call attention only to those which have a direct bearing upon the work at hand. The following lines are taken up in the present investigation, a preliminary report of which is given in the following pages:

1. Active protozoan fauna in the soil.
2. Numbers and types of protozoa in different soils at different depths, as shown by cultural methods.
3. Effect of protozoa on bacterial numbers and their decomposition of organic matter in the soil.

## ACTIVITY OF PROTOZOA IN THE SOIL.

Many investigators, from Ehrenberg (4) and Rosenberg Lipinsky (13) down to the present time, have tried to find out whether the protozoa are present in the soil in a trophic condition, or are encysted temporarily in the soil. The latest investigations seem to contradict one another as to the very fact of the occurrence of living protozoa in soils under normal conditions of temperature and moisture. Goodey (6) maintains that ciliated protozoa exist in the soil only in an encysted condition. Where free water was to be found in the soil, the presence of active protozoa could be demonstrated. However, he worked only with the ciliates, which, as large organisms, are not likely to be found active in soils having comparatively low moisture contents. Francé (5) states that from March to October the protozoa are active if other conditions are favorable; after this period they encyst because of the frost or aridity. Martin (10) found that smaller amoebae and flagellates play the most important rôle in the phenomenon of sick soils, and that the most common limiting factor affecting the activity of protozoa in the soil is the average quantity of water. Only recently Martin and Lewin (11) have shown that the dominant active fauna of the soil, as shown by the fresh films, consists mostly of amoebae, thecamoebae and small flagellates. Koch (9), who has probably done the most recent work on the occurrence of living protozoa in the soil, concluded that living protozoa do not seem to be present in field soils with an unusual moisture content. He made his observations by placing several drops of sterile water on a clean slide, and stirring into the water by means

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of a stirring rod a portion of soil to be examined. The examinations were made under the low power and for a period of not more than two minutes. There was very little chance for the observer to find the small flagellates among the soil particles. The motility of the protozoa is much slower in the soil than in culture solution, and it would be difficult to detect other than ciliates with the low power in such a short period.

To be able to get an idea of the time that is actually required for the protozoa to come out of encystment, the writer placed some soil in a deep cavity of a slide; the soil was covered with sterile tap water and examinations were made every five minutes for the detection of living forms of protozoa. After many repeated trials no flagellates could be found in a living condition before sixteen minutes, during which period the soil was covered with water. The first living ciliate was found only after the soil had been standing in contact with the water for 62 minutes. This led to the conclusion that there is no danger in extending the period of examination for active forms of protozoa to from 5 to 6 minutes. This length of time allowed closer observation among the soil particles, and a more thorough examination of the field. The high power (4 mm.) was used in case of doubt whether the organism was a small flagellate or a motile bacterium.

The error made in limiting the time of examination to only two minutes is made clear by the fact that in the 6 out of 20 greenhouse soils where Koch found protozoa, the organisms recorded are in five cases ciliates and amoebae, and only in one case flagellates. As was shown by Sherman (16), Cunningham (3), by the writer, and others, the number of flagellates is much larger than the number of ciliates, and we would expect that under conditions where moisture is the limiting factor, the smaller organisms would be able to lead an active life at a lower moisture content than the larger ones. Koch has also indicated that the moisture content of the soil is a primary limiting factor in the activity of the protozoa, while the texture and organic matter are secondary ones. It appears somewhat inconsistent, then, when one notes that he did not find any active protozoa at 34 and 36 per cent of moisture but did find them at 22.5 and 25.6 per cent in his examinations of greenhouse soils.

There is no doubt that there is a minimum moisture content for each soil below which protozoa cannot be found in a living condition. There must be another factor which is of greater importance than moisture, since this latter factor does not hold true with all the soils. Martin and Lewin (6), in addition to suggesting the use of fresh fixed films which bring out the active fauna of the soil, also used the method of adding water to the soil. In their examinations they were able to detect living organisms in different soils, but these were limited to small flagellates, amoebae and thecamoebae.

For this work the following soils were selected: (1) Sassafras loam rich in humus, and recently used for garden crops; (2) Sassafras loam poor in humus, in orchard; (3) Alloway clay, fairly rich in humus, in timothy; (4) Penn loam, in a variety of crops. Examinations were made every day for ten days in succession, during which period several rains occurred, some of which lasted a whole day. The moisture content was determined by air-drying 100 gm. of the soil. Several examinations were made with the low power (16 mm.) for a period of from 5 to 6 minutes. The high power was used to confirm the results in case of doubt.

The results of the examinations are set forth in Table I.

TABLE I.  
NUMBERS OF ACTIVE PROTOZOA IN THE SOIL.

Days of Examination	July 15, 1915 1st day		2d day		3d day		4th day		5th day		6th day		7th day		8th day		9th day		10th day	
	M.	Pr.	M.	Pr.	M.	Pr.	M.	Pr.	M.	Pr.	M.	Pr.	M.	Pr.	M.	Pr.	M.	Pr.	M.	Pr.
Humus	9.	....	9.5	....	13.5	S.F.	12.5	S.F.	13.	S.F.	10.	....	9.5	....	9.5	....	11.	....	14.	S.F.
Orchard	10.	....	9.5	....	12.	....	12.5	....	12.5	S.F.	9.	....	9.	....	9.	....	10.5	....	11.5	....
Timothy	14.	....	14.2	....	15.8	....	15.5	....	15.	....	14.	....	14.	....	13.5	....	15.	....	15.5	....
Penn Lm.	18.	....	18.	....	18.6	....	19.	....	20.	....	19.	....	18.	....	18.5	....	19.	....	20.	....

M = moisture in per cent.

Pr. = Protozoa.

S. F. = Small flagellates.

Glancing through the table one can readily see that the moisture content seems to be a limiting factor in the activity of protozoa in the soil. Small flagellates were found in the case of the rich humus soil at 13 to 14 per cent of moisture, which is the physical optimum for that soil. But no protozoa were found in the other soils except in one case, namely, in the orchard soil, where small flagellates were detected on the fifth examination. No protozoa were found by this method of examination to be active in the two heavy soils, even at a moisture content of 15.8 to 20 per cent. This tends to show that the moisture content of the soil is not the only limiting factor of the activity of protozoa, but that the structure and humus content of the soil likewise play an important part. Of the first two soils which are of the same physical texture and lie close to one another, the one rich in humus has shown greater protozoan activity than the other. Comparing the first two and the last two soils, we see that the lighter soils have shown some protozoan activity, while with the heavier soils no activity could be detected under correspondingly similar conditions of moisture by the same methods of examination. The protozoa found in the soils were all flagellates; had the moisture content of the soils been higher, no doubt ciliates also would have been found, because those soils develop ciliates in the culture solution, as will be seen later. As to amoebae and thecamoebae, it is thought that the method of examination was probably unsatisfactory for the detection of those organisms, if there were any living.



To learn something about the other factors which may play a part in making the protozoa active in the soil, the following experiment was conducted. Three soils of different texture were used, which will be termed "sandy soil," "loam soil," and "clay soil." Fifty-gram portions were placed in Erlenmeyer flasks and plugged with cotton. To one-third of the flasks organic matter was added in the form of dried blood in 3 gm. portions; thus an added excess of organic matter would probably eliminate any variations in the organic content of these soils. Another third of the flasks were sterilized after the proper amount of moisture had been added. The physical optimum moisture content was determined for each soil, and sterile water added in portions amounting to one-half, one, one and one-half, and two times the optimum for each soil. To those portions which had been sterilized, cultures of protozoa were added. (These were five-day-old cultures made by inoculating sterile Lohnis' soil extract with soil.) All flasks were kept in the incubator at 25° C. Examinations were made after three, five, eight and twenty days respectively. The occurrence of protozoa is given in Table II.

TABLE II.  
DEVELOPMENT OF PROTOZOA IN THE SOIL.

Soil	Moisture	Soil sterilized	Unsterilized, 3 gm. dried blood	Check
Sandy	0.5 optimum	.....	.....	.....
"	1.0 "	.....	.....	.....
"	1.5 "	.....	S.F.	.....
"	2.0 "	F. S.C.	F. S.C.	.....
Loam	0.5 "	.....	.....	.....
"	1.0 "	.....	.....	.....
"	1.5 "	S.F.	S.F.	.....
"	2.0 "	F. S.C.	F.	.....
Clay	0.5 "	.....	.....	.....
"	1.0 "	.....	.....	.....
"	1.5 "	.....	.....	.....
"	2.0 "	F. S.C.	F. C.	.....

F. = Flagellates. S. F. = Small flagellates. C. = Ciliates. S. C. = Small ciliates.

In the table one sees that the protozoa were found to be active with high moisture only where the soils have been sterilized, or when some organic matter has been added to them. No protozoa were found in the untreated soils even with the highest moisture content when the soil was almost saturated. But where organic matter was added, or where the organic matter of the soil was made more available by sterilization under pressure, active protozoa were found at one and one-half times the optimum, in the case of sandy and loam soils, and at two times the optimum in the case of the heavy clay soil. This brings out the fact that the protozoa will become active in different soils under similar treatment at correspondingly different moisture contents. In a lighter soil the pro-

tozoa may develop in the presence of easily soluble plant food at one and one-half times the optimum, which is about 18 to 20 per cent moisture. In a heavy soil at that moisture condition, which may be 30 to 35 per cent, no protozoa will be found. This question of easily available plant food which can be utilized by the protozoa, and hence bring about their greater activity, will be taken up in the third part of this work.

As to the types of protozoa developing in the soil, we conclude that with the methods used, the small flagellates develop as soon as conditions become favorable; these are followed by large flagellates and small ciliates. Large ciliates appear only in a few cases where the moisture content of the soil is very high, or other conditions are exceptionally favorable for their development. This confirms the results of some of the other investigators, who have either proven, or else have expressed the opinion, that the small flagellates are probably the active protozoan fauna of the soil, when conditions are nearly normal. Sherman (16) has also expressed the opinion that the active protozoan fauna in most soils is restricted to the flagellates. He found that ciliates become active only in soils containing much more moisture than is required for flagellates. He also shows that when sterilized soil is inoculated with normal soil, the protozoan fauna rises in numbers above that of normal soils, just as does the bacterial flora. Sterilization of the soil and the addition of organic matter seem to act in the same manner as an increase in the moisture content.

The method used seems to be fairly accurate for the detection of flagellates and ciliates in the soil, but it is of very little value for the detection of amoebae. The picric acid method, as outlined by Martin and Lewin (11) is being used for that purpose. Some organisms have been found by this method, which have not been detected in the soil by the ordinary water method; these resemble the thecamoebae described by the previously named writers. Not enough study has yet been made by this method to make it possible to state any definite results.

## II.—NUMBERS AND TYPES OF PROTOZOA IN THE SOIL.

### 1. *Numbers of Protozoa.*

The dilution method has been used for this part of the work. This was mentioned by Rahn (12) and more fully described by Cunningham (3) for the determination of total numbers of protozoa in the soil. Since all the protozoa do not develop on one medium, and as it is very hard to differentiate in dilution work between cysts and active forms, the total numbers of both cysts and active forms are given. Fifty cubic centimeters of sterile Lohnis' soil extract, in Erlenmeyer flasks, was inoculated with proper dilutions of soil and allowed to stand for five to ten days, then determinations of the presence of protozoa were made. Dilutions made were as follows: 1.0, 0.1, 0.01, 0.001, 0.002, 0.005, 0.0001. Soils used for this

part of the experiment as well as for the second are, 1, 2, 3, soil types used in the previous part of the work, and 4, a permanent forest soil. Samples were taken under sterile conditions, at depths of 1, 4, 8, 12, 20 and 30 inches.

The results are reported in Table III.

TABLE III.  
NUMBERS OF PROTOZOA AT DIFFERENT SOIL DEPTHS.

Depth of soil in inches	Soil A				Soil B			
	Protozoa	Moist'e	C%	N%	Protozoa	Moist'e	C%	N%
1	2,000-5,000 F.	9.3	1.56	0.1127	1,000-2,000 F.	9.0	.90	0.1158
4	2,000-5,000 F.	9.4	1.41	0.1176	100-1,000 F.	10.3	.41	0.1071
8	2,000-5,000 F.	9.0	1.76	0.0973	100-1,000 F.	10.0	.44	0.1036
12	10-100 F.	8.0	.57	0.0581	10-100 F.	8.3	.41	0.0819
20	0	9.0	.63	0.0420	0	6.0	.18	0.0413
30	0	10.0	.67	0.0581	0	7.7	.17	0.0287

Depth of soil in inches	Soil C				Soil D			
	Protozoa	Moist'e	C%	N%	Protozoa	Moist'e	C%	N%
1	5,000-10,000 F.	17.3	1.70	0.1918	10-100 F.	23.6	3.38	0.2345
4	1,000-2,000 F.	12.3	1.12	0.1596	10-100 F.	15.3	1.58	0.1015
8	100-1,000 F.	12.3	1.05	0.1267	1-10 F.	10.	0.56	0.0469
12	10-100 F.	11.3	.53	0.1050	0	11.	0.23	0.0294
20	0	12.3	.20	0.0518	0	14.	0.09	0.0294
30	0	13.3	.20	0.0350	0	14.5	0.12	0.0307

F. = Flagellates.

C% = Total carbon in per cent.

N% = Total nitrogen in per cent.

It is seen from the above table that protozoa are found in the soil between the depths of 1 and 12 inches, the largest numbers occurring just below the surface. The numbers decrease with the depth, and below 12 inches the soil is almost free from protozoa. At times protozoa were found when soil from 20 or even 30 inches below the surface was inoculated into the culture medium, but these cases were exceptional and should not be taken into account. Soil No. 4, which is an acid forest soil, seems to be very poor in numbers. Whether this is due to acidity or other reasons is not known. Ciliates and amoebae are not recorded as they occurred only occasionally. They usually varied from 10 to 100 per gram of soil, and their occurrence as to types will be pointed out later. These results seem to confirm Rahn (12), who found 1,000 to 10,000 flagellates and about 100 ciliates per gram of soil.

## 2. Types of Protozoa in the Soil.

The same soils and the same depths of sampling were used in this experiment. One gram of soil was inoculated into 50 c.c. of sterile soil extract, and records taken after 3, 5, 7, and 12 days incubation. In this part of the work only the types of protozoa developing in the solution will

be given. Details as to the different types of protozoa at different depths of soil and in different soils cannot be given as yet, but from the data collected it is seen that there is a difference among protozoan types in the different soil layers. The ciliates and large flagellates are found in greatest numbers at a depth of 4 inches, while the flagellates are present in largest numbers at a depth of 1 inch. It appears as if the smaller organisms are present in largest possible numbers near the surface, where conditions favor microörganic activities, while the large organisms (e. g. ciliates) are found at a somewhat lower depth, where the moisture conditions may be more favorable for their development. The common types found among the flagellates are *Monas guttula* Ehrbg., *Monas vivipara* Ehrbg., *Bodo ovatus* Stein, *Bodo augustus* Duj., *Chlamydomonas* and *Pleuromonas* types, more seldom the *Euglena viridis*, *Phyllomitus undulans*, etc. Among the ciliates: *Colpidium colpoda* Ehrbg., *Colpoda cucullis* O. F. M., and *Enchelys pupa* Ehrbg., most common ones, then *Prorodon ovum* Ehrbg., *Nassula elegans* Ehrbg., *Glaucoma* types, *Paramoecium*, *Pleuronema*, *Halteria*, *Uroleptus*, *Uronema*, *Strombidium*, *Chilodon*, *Oxytricha*, *Vorticella*, *Pleurotricha*, *Euplotes*, and several others.

The data on the occurrence of amoebae are very meagre because the methods used until now could not bring out the actual facts about those organisms. However, work is being done at the present time on that group of organisms.

### III.—EFFECT OF PROTOZOA ON BACTERIAL NUMBERS AND UPON AMMONIFICATION IN THE SOIL.

Russell and Hutchinson (11), after a series of experiments with partially sterilized soils, concluded that the bacterial numbers are limited in certain soils by detrimental organisms, which have much in common with protozoa; they also concluded that an increase in the rate of production of ammonia does not take place without bacterial multiplication; and since protozoan activities limit the bacterial numbers in the soil, they also limit the production of ammonia. Cunningham (3) has conducted experiments on "The Influence of Protozoa on the Numbers of Bacteria Developing in Ammonifying Solutions." Into 1 per cent bloodmeal solution plus .05 per cent  $K_2HPO_4$  he inoculated bacteria and protozoa from a culture of protozoa from the soil, and bacteria alone. Counts of the bacteria were made at intervals, and the results show that the soil protozoa, in solutions at all events, exercise a very decided limiting effect on the numbers of bacteria. He determined also the ammonia formed in the solutions with and without protozoa. In one instance no appreciable difference was found in the amount of ammonia formed; he attempts to explain that lack of difference, by the higher original bacterial count of the protozoa cultures. In the other two instances the ammonia accumulated in the pres-

ence of protozoa was smaller than in the solutions where they were absent, although the number of bacteria inoculated into the medium was greater in the case where they were with protozoa. By inoculating protozoan cultures and protozoa-free cultures of bacteria into partially sterilized soil, Cunningham found a great reduction in bacterial numbers where protozoa had been added. From these results he concluded that the presence of protozoa is the limiting factor, or at least one limiting factor, in bacterial numbers and activities in the soil.

The interaction of the bacteria and protozoa in the soil and the ammonia accumulation resulting from the decomposition of the organic matter is not such an easy problem as one might at first regard it. The different kinds of protozoa and bacteria, the type of soil, the treatment of the soil, the amount of moisture present, are all important factors in determining the ammonia accumulated in a given soil, which has been inoculated with protozoa and bacteria. One of the most obvious questions is, How does moisture influence the interaction between protozoa and bacteria? As it was shown in the early part of the work, moisture plays a very important part in the activity of protozoa; below a certain moisture content the protozoa will not be active (at least the author has not been able to find it so). The more moisture added to the soil, the more favorable the conditions become for the growth of protozoa. Protozoa develop best where the soil is fully saturated with water. If protozoa limit bacterial numbers and their activities in the soil, resulting in a decrease in ammonia accumulation, the amount of moisture added to a certain soil inoculated with a certain number of protozoa and bacteria, ought to influence the bacterial numbers and the amount of ammonia accumulated in the soil. By starting with a soil of low moisture content and adding moisture to it, the conditions should be made less favorable for bacterial development and ammonia accumulation where the protozoa are present, than where they are absent.

**METHODS**—100 gm. of air-dried soil (Sassafras loam was used throughout, with a physical optimum moisture of 15 per cent) and 155 mg. of nitrogen in the form of dried blood were placed in 250 c.c. Erlenmeyer flasks, and the proper amount of water added. The flasks were plugged and sterilized at 15 pounds for 15 minutes. One-c.c. portions from bacterial cultures free from protozoa and bacteria plus protozoa cultures were added to the flasks, which were then incubated for 7 days at 22° C. The culture of protozoa plus bacteria was made by inoculating some soil into Löhnis' soil extract. Protozoa-free cultures of bacteria were made by inoculating soil taken 20 inches deep and free from protozoa. Cultures were kept from 8 to 10 days and before being used for inoculation they were carefully examined as to the presence or absence of protozoa. The protozoa were counted with the microscope by the direct counting method,

and were found to be 100,000 to 200,000 per c.c. The numbers of bacteria were determined by plating out on Brown's albumen agar. The ammonia was determined by distilling the soil with MgO. The results are shown in Table IV.

TABLE IV.  
Mg. OF NITROGEN ACCUMULATED AS AMMONIA.\*

Inoculum taken from	Moisture in per cent	Bacteria alone			Protozoa + Bacteria		
		Bacteria per c.c. Inoculum	Mg. N as Ammonia	Average	Bacteria per c.c. Inoculum	Mg. N as Ammonia	Average
Soil A	16.	17,200,000	34.01 29.31	31.66	35,000,000	27.12 20.90	24.02
"	32.	"	36.22 38.13	37.18	"	58.04 50.12	54.08
Soil B	16.	32,500,000	16.38 13.58	14.98	13,000,000	17.70 15.64	16.67
"	32.	"	14.76 15.93	15.35	"	30.28 24.38	27.33

One can see from the above table that the increase in moisture gives an increase in ammonia production in both cases, but where the protozoa are present the results are somewhat striking. One might have expected that with an increase in moisture the activity of the protozoa would check the bacterial activities, but the results do not verify that. With soil A twice as many bacteria have been added to the soil in the protozoa culture as in the protozoa-free culture, and though at 16 per cent moisture the ammonia produced is less where the protozoa were present than where they were absent, at 32 per cent moisture the ammonia accumulated in the presence of protozoa is more than twice as great as with 16 per cent moisture, while the bacteria alone gave only a slight increase with the increase in the moisture content of the soil. The same holds true with the soil portions inoculated with the organisms isolated from soil B (unfertilized Sassafras loam), but in this case fewer bacteria had been added to the soil in the protozoa cultures. The soil portions, before the ammonia has been distilled off, were examined for living protozoa; small flagellates were found in an active state at the lower moisture content of the soil, and both ciliates and flagellates were found at the higher moisture content. It appears that the activities of protozoa stimulated the decomposition of organic matter in the soil, when the conditions were favorable for their development.

\* In this experiment as well as in all the subsequent experiments a check was used, consisting of the same amount of soil with the same quantity of organic matter, but not inoculated. This has been kept under the same conditions of moisture and temperature with the inoculated soil portions. The check, giving 2 to 3 mg. N, was always subtracted from the determinations, in order to eliminate the ammonia accumulated due to the decomposition of the organic matter by sterilizing the soil.

To be able to study more closely the effect of moisture upon the activities of the microorganisms in the soil, another experiment was started, in which different degrees of moisture were used. The same soil and organic matter and the same incubation period were used in the following experiment as in the previous one.

TABLE V.  
Mg. OF NITROGEN AS AMMONIA IN THE SOIL.

Inoculum taken from	Moisture in per cent	Bacteria alone			Protozoa + Bacteria		
		Bacteria per c.c. Inoculum	Mg. N as Ammonia	Average	Bacteria per c.c. Inoculum	Mg. N as Ammonia	Average
Soil A	7.	4,000,000	3.09 2.79	2.94	7,200,000	3.09 3.68	3.38
"	14.	"	6.91 6.91	6.91	"	9.11 10.88	10.00
"	28.	"	38.22 30.42	34.32	"	30.22 25.64	27.93
"	42.	"	3.23 4.41	3.82	"	3.97 4.70	4.34
Soil B	7.	18,000,000	2.06 2.35	2.21	28,000,000	2.06 1.76	1.91
"	14.	"	10.44 9.12	9.78	"	20.93 16.80	18.87
"	28.	"	16.02 13.82	14.92	"	24.55 23.23	23.89
"	42.	"	2.35 2.57	2.46	"	14.60 17.64	16.12

Table V shows that the presence of protozoa not only did not decrease the ammonification by bacteria when the moisture conditions became favorable for their development, but in some instances favored it. In looking through the columns one can see a parallel increase in ammonia accumulation as the moisture was increased from 7 per cent to 28 per cent, then the amount of ammonia accumulated drops down in all instances because at 42 per cent there was a free layer of water on the surface of the soil. The soils were examined for their protozoa content. No living protozoa were found in the soil that contained the 7 per cent moisture, small flagellates at 14 per cent, flagellates and ciliates at 28 per cent, flagellates and small ciliates at 42 per cent. With the increase in moisture content the protozoa became more active, but they did not seem to influence the amount of ammonia accumulated, and if they did have any influence, it was only beneficial.

One c.c. of inoculum was used in the experiments previously cited.

To see whether the numbers of bacteria and protozoa added might have any influence upon the ammonia accumulated, it was thought advisable to double the bacterial or protozoan numbers. Bacterial numbers in the soils were determined at the end of the seven-day incubation period by plating out the desired dilution on Brown's albumen agar. (2)

TABLE VI.

Mg. OF NITROGEN AS AMMONIA AND NUMBERS OF BACTERIA IN THE SOIL.

Moisture in per cent	Inoculum	Bacteria alone 12,000,000 bacteria per c.c. Inoculum				Protozoa + Bacteria 13,000,000 bacteria per c.c. Inoculum			
		N as Ammonia		Bacteria per gm. of soil		N as Ammonia		Bacteria per gm. of soil	
		Mg. N	Average	Nos. found	Average	Mg. N	Average	Nos. found	Average
7	1 c.c.	2.65		34,000,000		3.53		28,000,000	
		2.79	2.72	38,000,000	36,000,000	3.67	3.60	24,000,000	26,000,000
7	2 c.c.	2.94				3.38			
		2.82	2.88	.....	.....	3.24	3.31	.....	.....
14	1 c.c.	11.91		500,000,000		15.32		472,000,000	
		11.31	11.61	526,000,000	513,000,000	18.93	17.13	512,000,000	494,000,000
14	2 c.c.	14.70				23.67			
		15.28	14.99	.....	.....	19.99	21.83	.....	.....
28	1 c.c.	40.28		450,000,000		30.30		230,000,000	
		39.98	40.13	470,000,000	460,000,000	37.04	33.67	156,000,000	193,000,000
28	2 c.c.	37.19				33.22			
		40.72	38.96	.....	.....	31.32	32.27	.....	.....
42	1 c.c.	5.00		50,000,000		14.99		110,000,000	
		5.00	5.00	44,000,000	47,000,000	15.29	15.14	112,000,000	111,000,000
42	2 c.c.	5.73				15.29			
		4.56	5.15	.....	.....	14.85	15.07	.....	.....

The addition of double portions of the inoculum did not influence the results appreciably. In a few cases there is a slight increase in the ammonia accumulated, while in other cases there is a slight decrease. The presence of protozoa did not influence the ammonification in the soil with the increase in moisture. The records of bacterial numbers show an increase at from 7 per cent to 14 per cent of moisture, a slight decrease at 28 per cent, and a drop at 42 per cent. The drop between 14 per cent and 28 per cent is much greater in the cultures where protozoa were present. It is interesting to note that in both cases the amounts of ammonia produced do not correspond to the bacterial numbers; the largest bacterial numbers are found in both cases at 14 per cent of moisture, while the highest ammonia accumulation is found in both cases at 28 per cent.

In all the previous experiments the bacteria used for protozoa-free



culture were taken from the subsoil, and though the results are to be compared only within the same inoculum under varying moisture conditions, some objections might be raised to the use of different types of bacteria from those used in protozoa cultures. To eliminate this objection, fresh cultures were prepared by inoculating into 100 c.c. of soil extract 1 gm. and 0.00001 gm. of soil respectively. The cultures were examined after several days. Those that were inoculated with 1 gm. of soil contained bacteria and protozoa; those that were inoculated with 0.00001 gm. contained only bacteria. These cultures were now used for the work. Objections have also been raised by Russell (14) against the seven-day period of ammonification used by Lipman and his associates; he claimed that the results of the activities of the protozoa (e. g. detrimental factor) cannot be seen in such a short period of time. The following experiments were carried out for 14 and 30 days, while bacterial counts were taken 5, 7, 14 and 30 days after incubation. Though only .00001 gm. of soil was used for inoculation for the protozoa-free culture, the culture contained after 6 days 161,000,000 bacteria per c.c. as compared with 6,500,000 bacteria per c.c. where 1 gm. of soil was used for incubation. This great reduction in bacterial numbers might be due to the presence of protozoa in the second culture. The same amounts of soil and organic matter as in the previous experiments and 1 c.c. inoculum have been used in the following experiment.

TABLE VII.  
Mg. OF NITROGEN AS AMMONIA IN THE SOIL.

Moisture in per cent	Incubation	Bacteria 161,000,000 per c.c.		Protozoa + Bacteria 6,500,000 bacteria per c.c.	
		Mg. N	Average	Mg. N	Average
7	14 days	5.09	6.71	7.44	7.00
		8.32		6.56	
14	14 days	74.61	76.38	73.29	77.92
		78.15		82.55	
28	14 days	76.38	77.04	87.85	86.97
		77.70		86.08	
42	14 days	10.38	10.20	9.35	10.53
		10.02		11.70	
7	30 days	14.35	17.36	29.64	27.06
		20.37		24.48	
14	30 days	72.41	71.31	67.56	68.44
		70.21		69.32	
28	30 days	76.82	78.13	76.09	75.58
		79.44		75.06	
42	30 days	7.00	6.26	15.67	14.35
		5.52		13.02	

Table VII gives the ammonia that had accumulated in the cultures after 14 and 30 days respectively. The facts brought out in the previous experiments hold true also in this one; the increase in moisture gave a higher ammonia accumulation up to double the optimum, the moisture that resulted in a decrease. The presence of protozoa did not affect in any way the ammonia accumulated in the cultures. But turning to Table VIII, where the bacterial numbers of the same cultures are given for different periods, one finds a great difference between the cultures containing protozoa and those free from those organisms. The protozoa-free cultures contained after a five-day period of incubation 18 to 325 millions per gram, the numbers varying with the moisture content of the soil, the highest numbers being found with 28 per cent of moisture, and the lowest with 7 per cent. After 7 days inoculation, the bacterial numbers greatly increased, the highest numbers, 1536 millions per gram of soil, occurring with 14 per cent moisture and the lowest with 42 per cent. After the seven-day period the bacterial numbers decreased steadily, but the relation between the different moistures holds true also with the fourteen- and thirty-day periods: the highest bacterial numbers occurring with 14 per cent or 28 per cent, the lowest with 7 per cent or 42 per cent.

TABLE VIII.  
BACTERIAL NUMBERS IN THE SOIL, IN MILLIONS PER GRAM.

Moisture in per cent	Bacteria alone used as Inoculum								Protozoa + Bacteria							
	5 days		7 days		14 days		30 days		5 days		7 days		14 days		30 days	
	Bacteria	Average	Bacteria	Average	Bacteria	Average	Bacteria	Average	Bacteria	Average	Bacteria	Average	Bacteria	Average	Bacteria	Average
7	14.		596.		612.		30.		176.		204.		260.		440.	
	22.	18.	582.	589.	604.	608.	33.	31.5	186.	181.	226.	215.	224.	242.	530.	485.
14	26.		1580.		828.		100.		148.		298.		138.		74.	
	36.	31.	1492.	1536.	638.	730.	90.	95.	144.	146.	312.	305.	152.	145.	46.	60.
28	314.		972.		885.		60.		102.		142.		162.		54.	
	336.	325.	878.	925.	864.	875.	72.	66.	86.	94.	128.	135.	124.	142.	60.	57.
42	216.		320.		660.		28.		42.		36.		108.		42.	
	206.	211.	296.	308.	685.	673.	28.	28.	48.	45.	28.	32.	102.	105.	52.	47.

When the bacterial numbers of the protozoa plus bacteria cultures are compared with those of the protozoa-free culture, a great difference is found; while the latter contained the highest bacterial numbers at the optimum moisture conditions which seemed to vary between 14 per cent and 28 per cent, and the lowest numbers with the 7 per cent and 42 per cent of moisture; where the protozoa were present the bacterial numbers were highest with 7 per cent moisture, and only in one case with 14 per

cent; the numbers of bacteria then rapidly decreasing with the increase in moisture. The 7 per cent moisture content should have given the lowest bacterial numbers as one could have expected from the results of the ammonia accumulation and the bacterial numbers in the protozoa-free cultures. It seems as if the increase in moisture, making the conditions more favorable for the protozoa development, had, at the same time, a detrimental effect upon the bacterial numbers. Upon examining the soils with a microscope, the protozoa were not found in an active state with the 7 per cent moisture, but were found leading a trophic life in cultures containing 14 per cent moisture and more.

This experiment seems to bear out the conclusions of Russell and Hutchinson that the protozoa are one of the factors detrimental to bacterial numbers. But it does not bear out their second conclusion, that the increase in ammonia production was due to the destruction of the detrimental organisms and the rise in bacterial numbers. Although the bacterial numbers were greatly decreased in the presence of protozoa when the conditions became favorable for their development, the ammonification was not affected at all. It appears that either the bacteria destroyed played no part at all in the ammonia accumulation, or that the protozoa, or some types of protozoa, took part in the decomposition of the organic matter and the accumulation of ammonia. A suggestion with respect to the possible ammonification by protozoa was made in 1896 by Bréal (14).

To see whether the presence of protozoa can have any effect at all upon bacteria having a strong ammonifying power, the following experiment was started. A series of flasks containing 100 gm. of soil, 155 mg. of nitrogen as dried blood, and different quantities of moisture, were sterilized, then inoculated with 1 c.c. of a three-day-old culture of *B. mycoides* and protozoa plus bacteria culture grown for 10 days in soil extract. The flasks were incubated at 22° to 25° C, and duplicate ammonia determinations made after 6, 20 and 40 days.

The presence of protozoa not only did not decrease the ammonia accumulated by the *B. mycoides*, but in some instances a strong associative action between the different organisms is found, and the protozoa, at least as far as ammonification is concerned, do not have any detrimental effect at all.

Russell (14) in his answer to his American critics states again, "The increased ammonia production is attributed to the increased numbers of bacteria. The factor limiting bacterial numbers in ordinary soils is not bacterial, nor is it any product of bacterial activity, nor does it arise spontaneously in soils. The effect is rather variable, but is usually most marked in moist soils that have been well supplied with organic manures. The detrimental organism develops more slowly than bacteria . . . and causes a marked reduction in the numbers of bacteria."

TABLE IX.  
Mg. OF NITROGEN AS AMMONIA IN THE SOIL.

Moisture in per cent	Incuba- tion	B. mycoides		Protozoa + Bacteria		B. mycoides + Protozoa	
		Mg. N	Average	Mg. N	Average	Mg. N	Average
10	6 days	3.48	3.40	0.82	0.97	2.29	2.51
		3.32		1.12		2.73	
10	20 days	6.20	5.94	0.38	0.20	8.91	8.97
		5.67		0.02		9.02	
10	40 days	6.26	6.56	5.82	4.94	10.97	12.22
		6.85		4.06		13.46	
25	6 days	4.21	4.58	2.00	2.30	5.53	6.18
		4.94		2.59		6.82	
25	20 days	14.93	15.09	15.61	15.82	60.79	59.84
		15.24		16.03		58.89	
25	40 days	76.67	78.58	89.90	89.36	94.02	93.74
		80.49		88.82		93.46	

These conclusions have to be taken with a great deal of care in the light of the present experiments. The results brought out in this paper show that though the protozoa may be detrimental to bacterial numbers, they do not influence the ammonia accumulated in the soil, a fact which is the important part of the question, and with which we concern ourselves in studying the problems of soil fertility. The question whether the protozoa do play any part in the fertility of the soil can be answered only after sufficient work has been done with protozoa in their interaction with the soil bacteria.

The presence of protozoa seems to be detrimental to bacterial numbers. But, either the bacteria destroyed do not take any active part in the ammonification, or the protozoa, destroying some bacteria, influence beneficially the decomposition of organic matter. Culture solutions containing protozoa have a more pleasant odor than those containing bacteria alone; it appears as if the protozoa either destroy the decomposition products or the putrefactive organisms. These facts lead to the following question, "Are not the protozoa natural and necessary factors in the fertility of the soil?"

Since the completion of this work for publication, the issue of the Experiment Station Record, Vol. XXXIII, No. 6, has come to hand. This contains abstracts of articles dealing in one way or another with soil protozoa. This work has been done by two of the first and foremost students on protozoa and their relation to bacterial activities. These are Goodey and Hutchinson. In his "Investigations on Protozoa in Relation to the Factor Limiting Bacterial Activity in the Soil," Goodey (7) comes to the follow-

ing conclusions: "The protozoa, including ciliates, amoebae and flagellates added to the soil have not been able to act as a factor limiting bacterial activity in the soil. Inferentially, therefore, the ciliates, amoebae, and flagellates obtainable from ordinary soil under cultural conditions do not function as the limiting factor." Hutchinson (8) in his work on green manures says: "The rapid ammonification which takes place when green manure is placed in water and allowed to ferment was found to be accompanied by the development of large numbers of ciliates, flagellates, and amoebae, whose presence does not appear in this instance to be prejudicial to the activity of ammonifying bacteria."

But if the protozoa play a doubtful part in the fertility of normal soils, might not they play an important rôle in specialized soils, which may become "sick" or "tired" after being subjected to the same treatment for a number of years? In those soils certain types of protozoa might develop which would become detrimental factors in the fertility of those soils. At the suggestion of Dr. Löhnis, the writer has undertaken to create the "sick" or "tired" condition of the soil artificially in the laboratory by treating soils with high quantities of manure, keeping them under wide ranges of moisture and temperature, and studying the types and activities of the protozoa that develop under those conditions.

The work last outlined, together with the isolation of different types of protozoa free from bacteria, and the working out of different methods for the study of protozoa in the soil, form the main lines of present investigations by the writer, in the field of soil protozoology.

#### SUMMARY.

1. Moisture, humus content and the structure of the soil are the important factors governing the activities of the protozoa.

2. Sterilization of soil and addition of easily soluble organic matter will make the conditions optimum for protozoan activities at lower moisture content than the corresponding unsterilized or untreated soils.

3. The flagellates are the most common soil protozoa, found active in the soil with moisture content too low for the development of the other groups.

4. The flagellates are the largest group of soil protozoa; the greatest number of flagellates are found in the soil just below the surface; the ciliates at a depth of 4 inches; the numbers decrease with the depth, so that below 12 inches the soil is practically free from protozoa.

5. Soil protozoa do not have any appreciable influence upon the ammonification by bacteria.

6. The presence of protozoa acts detrimentally upon bacterial numbers, so that when the conditions become favorable for protozoa development, the bacterial numbers decrease.

7. The detrimental effect of the protozoa upon the bacterial numbers and their non-detrimental, and even beneficial effect at times upon the soil, and their influence upon ammonification in the soil, might be explained by one of the following assumptions: (1) if the protozoa destroy bacteria, they destroy non-ammonifying organisms; (2) the protozoa themselves take part in the process of ammonification; or (3) the disintegration of the bacterial cells results in decomposition products which might be responsible for high ammonia production.

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## QUANTITATIVE MEDIA FOR THE ESTIMATION OF BACTERIA IN SOILS.\*

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A study of bacteriology in the estimation of the number of bacteria in soils seems to be of considerable value when taken together with other factors. Not only the number, but also the character of the organisms present, must exert greater or less influence upon soil fertility, and a study of both is essential to one's understanding of the problems involved.

Of the methods which have been devised for use in counting soil bacteria, the plate method is without doubt the most satisfactory. But the choice of a good medium is of prime importance. Among the numerous formulae suggested up to the present time there are few which are not seriously lacking in one or more respects. Quantitatively, the medium which will permit the development of the maximum number of colonies is usually most satisfactory. In order, too, that the results be comparable at different times and different places, it is desirable that the constituents be of definite chemical composition, or that the medium be made up synthetically. For qualitative work, however, definiteness of composition assumes less prominence and admits of greater choice in the selection of materials.

Recent contributions to the subject by Brown (1) and Conn (2) have indicated progress in both quantitative and qualitative directions. Earlier work by Temple (5), by Lipman and Brown (4), and by Fischer (3), forms a basis of comparison.

It was the purpose of these experiments, besides corroborating some of the work noted above, to contribute something toward the improvement of present facilities.

With this in mind, experimental work was outlined in which particular attention was directed to a comparison of a number of media which have been in use for some time in bacteriological studies. A few variations and additions were planned and carried out as noted below.

The comparisons of the various media were made from at least two different preparations upon several soils. One preparation of media or one or two soils do not seem to be a sufficient test upon which to base a correct interpretation of results. It is evident from a study of the tables following that not all soils behave toward the various media to a like extent, or even, at times, in the same direction in the comparative tests.

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The agar used throughout the experiments was thoroughly mixed so that the possibility of variation at this point was slight. In every case the nitrogenous material was added just before sterilization. All plates were poured in triplicate with dilutions of 60,000, and subsequently incubated at a temperature of about 22° C.

Six types of fresh soil, designated respectively by the Bureau of Soils as Penn Loam, Penn Sandy Loam, Sassafras Gravelly Loam, Sassafras Sandy Loam, Sassafras Silt Loam, and Alloway Clay, were used. In order to test the media upon widely varied soil conditions, samples were secured, so far as possible, from plots receiving different treatments.

TABLE I.  
COMPOSITION OF MEDIA USED IN THE COMPARATIVE TESTS.

MEDIA	Constituents	Liters Distilled Water	Liters Tap Water	Gm. Agar.	Gm. $K_2HPO_4$	Gm. $NH_4H_2PO_4$	Gm. $MgSO_4$	Gm. Dextrose	Gm. Peptone	Gm. Albumen	Gm. Casein	Gm. Urea	Gm. Asparagine	Gm. $CaCl_2$	Gm. KCl	Gm. $NH_4NO_3$	Gm. $FeCl_3$	Gm. $Fe_2(SO_4)_3$	Gm. Sod. Asparaginate	c.c. Hay Infusion	c.c. Bloodmeal Ext'ct
I. Lipman and Brown's Modified Synthetic Agar...	1.0	...	15	.5	...	.2	10	.05	...	...	...	...	...	...	...	...	...	Tr.	...	...	...
II. Conn's Sodium Asparaginate Agar°	1.0	...	12	...	1.5	.2	1	...	...	...	...	...	...	.1	.1	...	Tr.	...	.5	...	...
III. Brown's Urea Agar....	1.0	...	15	.5	...	.2	10	...	...	...	...	.05	...	...	...	...	...	Tr.	...	...	...
IV. Brown's Asparagine Agar	1.0	...	15	.5	...	.2	10	...	...	...	...	...	.05	...	...	...	...	Tr.	...	...	...
V. Brown's Casein Agar....	1.0	...	15	.5	...	.2	10	...	...	...	.1	...	...	...	...	...	...	Tr.	...	...	...
VI. Brown's Albumen Agar...	1.0	...	15	.5	...	.2	10	...	...	.1	...	...	...	...	...	...	...	Tr.	...	...	...
VII. Albumen Agar.....	1.0	...	15	.5	...	.2	10	...	...	.2	...	...	...	...	...	...	...	Tr.	...	...	...
VIII. Albumen Agar.....	1.0	...	15	.5	...	.2	10	...	...	.3	...	...	...	...	...	...	...	Tr.	...	...	...
IX. Albumen Agar.....	1.0	...	15	.5	...	.2	10	...	...	.4	...	...	...	...	...	...	...	Tr.	...	...	...
X. Bloodmeal Agar.....	.92	...	15	.5	...	.2	10	...	...	...	...	...	...	...	...	...	...	Tr.	...	...	80†
XI. Hay Infusion Agar.....	.94	...	15	.5	...	.2	10	...	...	...	...	...	...	...	...	...	...	Tr.	...	60†	...
XII. Temple's Peptone Agar.....	1.0	...	15	...	...	...	...	1	...	...	...	...	...	...	...	...	...	Tr.	...	...	...
XIII. Albumen Agar.....	1.0	...	15	.5	...	.2	10	...	...	.1*	...	...	...	...	...	...	...	Tr.	...	...	...
XIV. Albumen Agar.....	1.0	...	15	.5	...	.2	10	...	...	.2*	...	...	...	...	...	...	...	Tr.	...	...	...
XV. Albumen Agar.....	1.0	...	15	.5	...	.2	10	...	...	.3*	...	...	...	...	...	...	...	Tr.	...	...	...
XVI. Albumen Agar.....	1.0	...	15	.5	...	.2	10	...	...	.4*	...	...	...	...	...	...	...	Tr.	...	...	...
XVII. Urea-Ammonium Nitrate Agar.....	1.0	...	15	.5	...	.2	10	...	...	...	...	.05	...	...	...	.05	...	Tr.	...	...	...
XVIII. Urea-Ammonium Nitrate Agar.....	1.0	...	15	.5	...	.2	10	...	...	...	...	.05	...	...	...	.1	...	Tr.	...	...	...
XIX. Urea-Ammonium Nitrate Agar.....	1.0	...	15	.5	...	.2	10	...	...	...	...	.05	...	...	...	.5	...	Tr.	...	...	...
XX. Urea-Ammonium Nitrate Agar‡	1.0	...	15	.5	...	.2	10	...	...	...	...	.05	...	...	...	.1	...	Tr.	...	...	...

\* Dissolved in 1 c.c.  $\frac{N}{6}$  NaOH and 5 c.c. water.

\* Brought to .8% acid.

‡ Brought to .25% acid.

§ Prepared by digesting 10 gm. bloodmeal at 100° C. in 100 c.c. of water for one hour and filtering.

† Prepared by digesting 10 gm. of timothy hay in 100 c.c. of water at 100° C. for one hour and filtering.

Since the nature of the nitrogenous material seems to exert considerable influence upon the development of colonies (1), it was thought that possibly the substitution of certain new substances might be advantageous. A few preliminary trials with an extract of bloodmeal and hay infusion as a source of nitrogen had given promising results, but the variability in composition and difficulty of preparation made them unsatisfactory for use in quantitative media. For qualitative purposes they show up well and the differentiation in colony growth is pronounced. They were included in the first two tests.

The albumen agar suggested by Brown was subjected to some changes. For .1 gm. of egg albumen, as is recommended, .2 gm., .3 gm. and .4 gm. were substituted in an effort to determine if there might not be a point between .1 gm. and .5 gm. where a larger number of colonies might develop.

The composition of all media used in the experiments is given in the following table; except where otherwise noted, the reaction was not adjusted.

#### *Series No. 1.*

In the first series twelve media were compared on four soils, Lipman and Brown's modified synthetic agar being taken as a basis of comparison. Sterilization was accomplished in flowing steam for one hour and fifteen minutes.

A comparison of Tables II and III shows that there is a decided increase between the third and fifth day. While varying in extent with different media, this increase averages about 75 per cent. It should be observed also that the relative bacterial numbers as indicated by the counts on the third day is not quite the same as shown by the longer incubation period.

With one exception, Table III shows the sodium asparaginate agar to have given the highest counts. Brown's albumen agar, Temple's peptone agar and the bloodmeal agar having shown up well. With Soil No. 3 Brown's albumen agar stood first by a small margin. The differences between the various media are, however, less pronounced than might be expected. In fact it would be difficult to differentiate between some of them from the counts secured.

The rather inconsistent counts secured with the albumen agar may be due to lack of thorough distribution of the coagulated material even though the tubes were shaken before pouring the plates.

TABLE II.—THREE-DAY INCUBATION PERIOD.†  
MILLIONS OF BACTERIA PER GRAM OF AIR-DRY SOIL.\*

Media	Soil No. 1 Penn Loam	Soil No. 2 Penn Sandy Loam	Soil No. 3 Sassafras Sandy Loam	Soil No. 3 Alloway Clay
I. Lipman & Brown's Modified Synthetic Agar..	5.49	5.01	3.46	3.01
II. Conn's Sodium Asparaginate Agar .....	7.65	6.48	3.56	4.50
III. Brown's Urea Agar.....	6.12	4.09	3.43	3.84
IV. Brown's Asparagine Agar...	4.42	3.63	2.47	2.80
V. Brown's Casein Agar.....	6.64	6.11	3.44	4.68
VI. Brown's Albumen Agar....	6.77	5.53	3.47	4.87
VII. Albumen Agar.....	6.93	5.67	3.30	4.80
VIII. Albumen Agar.....	6.40	5.14	3.27	4.88
IX. Albumen Agar.....	6.77	5.86	3.35	4.83
X. Bloodmeal Agar.....	7.05	5.84	3.72	5.27
XI. Hay Infusion Agar.....	5.12	4.89	2.58	3.37
XII. Temple's Peptone Agar....	5.95	4.85	3.42	3.27

† For individual counts in all cases see corresponding tables at the end.

\* Calculated on assumed moisture content of fresh soil.

TABLE III.—FIVE-DAY INCUBATION PERIOD.  
MILLIONS OF BACTERIA PER GRAM OF AIR-DRY SOIL.

Media	Soil No. 1	Soil No. 2	Soil No. 3	Soil No. 4
I. Lipman & Brown's Modified Synthetic Agar.	9.28	7.80	7.22	6.32
II. Conn's Sodium Asparaginate Agar.....	11.98	9.58	7.09	8.23
III. Brown's Urea Agar.....	9.77	8.41	6.03	6.72
IV. Brown's Asparagine Agar...	8.64	6.86	4.71	5.07
V. Brown's Casein Agar.....	10.17	9.26	7.20	8.10
VI. Brown's Albumen Agar....	10.63	9.24	7.51	8.05
VII. Albumen Agar.....	10.15	9.12	7.22	7.96
VIII. Albumen Agar.....	10.37	9.11	7.04	8.09
IX. Albumen Agar.....	10.03	9.10	6.73	7.59
X. Bloodmeal Agar.....	11.64	9.24	6.99	8.14
XI. Hay Infusion Agar.....	9.69	7.78	6.26	5.94
XII. Temple's Peptone Agar....	10.82	8.47	7.25	6.74

### Series No. 2.

Series No. 2 was essentially the same as Series No. 1. However, sterilization of all media except the casein and albumen agars was accomplished under steam pressure of one atmosphere for 15 minutes, the casein and albumen agars having been sterilized as before. In an attempt to eliminate the difficulty due to the coagulation of the albumen, a solution of it in 1 c.c.  $\frac{N}{5}$  NaOH and 5 c.c. of water was made for media numbers XIII, XIV, XV and XVI. The addition of this solution caused no perceptible coagulation even upon sterilization, and it was found possible to add the material to the medium without cooling. This procedure greatly simplified the preparation of the albumen agar.

TABLE IV.  
MILLIONS OF BACTERIA PER GRAM OF AIR-DRY SOIL.

Media	Soil No. 5 Penn Sandy Loam	Soil No. 6 Alloway Clay	Soil No. 7 Sassafras Silt Loam	Soil No. 8 Penn Loam
I. Lipman & Brown's Modified Synthetic Agar.	6.29	6.93	3.62	11.83
II. Conn's Sodium Asparaginate Agar.....	5.95	8.87	4.59	12.23
III. Brown's Urea Agar.....	4.66	7.94	4.04	10.78
IV. Brown's Asparagine Agar...	3.98	6.02	3.63	9.89
V. Brown's Casein Agar.....	5.73	6.63	3.53	11.40
VI. Brown's Albumen Agar....	6.49	7.38	4.50	11.78
X. Bloodmeal Agar.....	6.94	7.92	3.71	11.50
XI. Hay Infusion Agar.....	4.83	7.61	3.02	9.46
XII. Temple's Peptone Agar....	6.63	7.29	3.70	11.95
XIII. Albumen Agar.....	6.98	8.82	4.37	12.10
XIV. Albumen Agar.....	7.04	8.57	4.21	11.63
XV. Albumen Agar.....	6.92	7.31	4.22	11.14
XVI. Albumen Agar.....	6.59	7.48	4.21	11.77

It is evident that the relationships shown in Series No. 1 are apparently the same in this, the different forms of sterilization having exerted no appreciable influence. The albumen agars do not seem to have deteriorated by the solution of the albumen in sodium hydroxide, but the counts are much less variable and slightly higher than with No. VI. No consistent differences appeared either here or in Series No. 1 between the media having respectively .1 gm., .2 gm., .3 gm., and .4 gm., of albumen.

*Series No. 3.*

Numbers III, IV, V, VII, VIII, IX, X, XI, XIV, XV and XVI did not warrant further investigation, and in Series No. 3 a comparison of five media was made. With the exception of Brown's albumen agar VI, all were sterilized at 15 pounds for fifteen minutes.

TABLE V.  
MILLIONS OF BACTERIA PER GRAM OF AIR-DRY SOIL.

Media	Soil No. 9 Penn Loam	Soil No. 10 Penn Sandy Loam	Soil No. 11 Sassafras Silt Loam	Soil No. 12 Alloway Clay
I. Lipman & Brown's Modified Synthetic Agar.	6.37	4.34	4.91	7.63
II. Conn's Sodium Asparaginate Agar.....	7.49	4.20	6.23	9.75
VI. Brown's Albumen Agar....	7.11	4.27	5.86	9.29
XII. Temple's Peptone Agar....	6.59	4.11	5.11	8.28
XIII. Albumen Agar.....	7.39	4.52	6.13	9.67

Here again the asparaginate agar gave the highest counts in three out of four cases, albumen agar XIII giving the highest count in one case. Moulds did not develop as readily upon the asparaginate agar as upon

most of the others tried and counting was rarely rendered difficult. However, there are such small differences between the asparaginate and albumen agars that the choice of one over the other would be determined by other factors than mere colony development. But apparently the use of sodium asparaginate will greatly limit the number of disturbing elements in plate counting.

At this point it seemed worth while to determine the effect of the introduction of several forms of nitrogen into a medium. It seems reasonable to expect that with the use of nitrogen from several sources, a larger number of bacteria might develop colonies. The use of  $\text{NH}_4\text{NO}_3$  suggested itself as furnishing two forms, and a third was taken in urea which had been observed in Tables III and IV to give comparatively high counts. Accordingly, the media XVII, XVIII, and XIX were made up and compared with asparaginate and albumen agars.

TABLE VI.  
MILLIONS OF BACTERIA PER GRAM OF AIR-DRY SOIL.

Media	Soil No. 13 Penn Loam	Soil No. 14 Alloway Clay	Soil No. 15 Sassafras Gravelly L'm	Soil No. 16 Sassafras Silt Loam
II. Sodium Asparaginate Agar.	15.97	7.35	3.55	9.88
XIII. Albumen Agar.....	15.50	7.73	2.68	8.95
XVII. Urea-Ammonium Nit. Agar	14.20	8.44	2.99	7.47
XVIII. Urea-Ammonium Nit. Agar	17.30	8.80	3.92	10.41
XIX. Urea-Ammonium Nit. Agar	15.23	7.40	2.55	8.95

Table VI shows that with the four soils used the urea-ammonium nitrate agar containing .05 gm. of urea and .1 gm. of ammonium nitrate gave higher counts than any of the other media. There was, however, a tendency toward mould development, and in an effort to discourage this, Series No. 5 was started in which the reaction of the urea-ammonium agar was changed to .2½ per cent acid in Medium No. XX.

TABLE VII.  
MILLIONS OF BACTERIA PER GRAM OF AIR-DRY SOIL.

Media	Soil No. 17 Penn Loam	Soil No. 18 Penn Sandy Loam	Soil No. 19 Sassafras Silt Loam	Soil No. 20 Alloway Clay
II. Sodium Asparaginate Agar.	13.57	6.48	2.21	5.03
XVIII. Urea-Ammonium Nit. Agar	16.02	7.10	2.42	4.89
XX. Urea-Ammonium Nit. Agar	14.95	7.04	3.15	4.98

### Series No. 5.

The change in the reaction did not seem to impair the efficiency of the urea-ammonium nitrate agar but rendered it decidedly less favorable to moulds. The relationship to the sodium asparaginate agar was again checked up, tending to show that the urea-ammonium nitrate agar will

permit the development of as many colonies as, or more than, the former; it is also easier to prepare and is much less expensive.

Tests of other combinations would no doubt result in the accumulation of valuable information, but it seems that the media question may be most satisfactorily solved by the use of differential media which will allow the development of particular species of organisms rather than by an attempt to determine the greatest number regardless of species or groups. This should give, in addition to relative bacterial numbers, certain indications concerning the biological activities in different soils.

#### SUMMARY.

The results of the present work indicate that:

1. Sodium asparaginate agar, albumen agar, and urea-ammonium nitrate agar will in most cases give a greater colony development than other media in common use for bacteriological work.
2. The albumen agar, in which the albumen is dissolved in NaOH, will give more consistent results than if the albumen is used in water solution.
3. A five-day incubation period gave considerably higher bacterial counts than a three-day incubation period.
4. Sterilization either by flowing steam or at a pressure of one atmosphere will give equally good results.
5. Differentiation in bloodmeal and hay infusion agars is marked.

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In the following tables the separate determinations are given, from which the averages set forth in the foregoing tables were derived.

TABLE II-A.  
MILLIONS OF BACTERIA PER GRAM OF AIR-DRY SOIL.

Media.	Soil No. 1			Soil No. 2			Soil No. 3			Soil No. 4		
I.	5.14	5.23	6.10	5.30	5.01	4.73	3.21	3.71	3.46	3.07	3.22	2.74
II.	7.32	8.01	7.61	6.82	6.62	5.99	3.76	3.39	3.52	5.66	4.18	3.66
III.	6.16	6.40	5.80	3.59	4.18	4.49	3.14	3.68	3.47	4.03	3.72	3.77
IV.	4.19	4.82	4.26	3.81	3.62	3.45	3.06	2.14	2.22	2.79	3.06	2.55
V.	6.62	6.59	6.91	6.77	5.18	6.37	3.55	3.16	3.62	4.69	5.27	4.09
VI.	6.99	6.03	7.29	5.61	4.88	6.11	3.01	3.82	3.59	4.96	5.53	4.11
VII.	7.30	7.00	6.50	5.19	6.27	5.55	2.98	3.46	3.45	5.18	4.17	5.06
VIII.	6.08	6.82	6.31	4.39	5.72	5.31	3.61	2.89	3.32	4.26	5.29	5.10
IX.	7.11	6.81	6.40	5.66	5.92	6.01	3.53	3.21	3.30	3.99	5.33	5.18
X.	7.24	6.82	7.08	6.77	5.14	5.62	3.49	3.76	3.92	5.17	5.38	5.26
XI.	5.02	5.45	4.90	4.97	4.38	5.33	2.95	2.20	2.60	2.98	3.30	3.82
XII.	6.40	6.04	5.42	5.08	4.39	5.08	3.41	3.02	3.82	3.06	3.66	3.10

TABLE III-A.  
MILLIONS OF BACTERIA PER GRAM OF AIR-DRY SOIL.

Media.	Soil No. 1			Soil No. 2			Soil No. 3			Soil No. 4		
I.	9.91	8.63	9.30	7.82	7.58	7.99	7.01	7.50	7.16	6.38	6.61	5.98
II.	12.46	12.01	11.46	9.64	9.22	9.89	6.93	7.01	7.33	8.52	8.21	7.96
III.	10.15	9.38	9.79	8.43	8.62	8.19	5.81	6.19	6.08	7.11	6.39	6.66
IV.	8.94	8.69	8.30	6.61	6.93	7.04	4.81	4.92	4.40	5.14	4.76	5.31
V.	9.96	10.36	10.18	9.22	9.60	8.96	7.10	7.03	7.48	8.32	7.94	8.01
VI.	10.10	11.02	10.76	9.11	10.32	8.28	7.09	7.51	7.92	7.08	8.39	8.69
VII.	9.75	10.40	10.31	9.29	8.51	9.56	7.21	7.03	7.42	7.49	7.88	8.50
VIII.	10.40	11.12	9.60	8.82	9.14	9.36	7.43	6.57	7.11	7.79	8.32	8.16
IX.	10.21	9.81	10.06	8.00	9.21	10.10	6.91	6.55	6.73	7.16	7.49	8.13
X.	11.90	11.34	11.68	9.42	9.31	8.99	7.01	7.19	6.77	7.82	8.17	8.42
XI.	9.95	9.62	9.49	8.36	8.01	6.98	6.31	6.42	6.06	6.22	6.56	5.03
XII.	10.81	10.42	11.24	8.71	8.49	8.22	7.23	7.07	7.46	7.10	6.82	6.30

TABLE IV-A.  
MILLIONS OF BACTERIA PER GRAM OF AIR-DRY SOIL.

Media.	Soil No. 5			Soil No. 6			Soil No. 7			Soil No. 8		
I.	5.49	7.17	6.21	6.95	7.16	6.69	3.67	3.41	3.79	12.20	12.40	10.89
II.	6.44	5.56	5.85	8.67	9.12	8.82	4.61	4.52	4.63	12.06	11.83	12.81
III.	5.11	4.61	4.25	8.36	7.95	7.51	4.03	4.11	3.98	11.10	10.76	10.48
IV.	3.74	4.23	3.97	5.93	6.12	6.02	3.61	3.70	3.58	10.62	9.41	9.63
V.	5.85	5.92	5.42	6.21	6.78	6.91	3.92	3.46	3.21	11.06	11.82	11.32
VI.	7.25	6.65	5.56	7.67	7.48	6.98	4.58	4.61	4.32	11.26	11.91	12.16
X.	6.65	6.86	7.31	8.15	7.95	7.65	3.91	3.76	3.47	10.90	12.13	11.46
XI.	5.03	4.34	5.12	8.05	7.32	7.46	2.80	3.22	3.03	9.01	9.48	9.88
XII.	7.41	6.45	6.02	6.90	7.22	7.76	3.72	3.81	3.56	12.62	11.83	11.40
XIII.	7.16	7.03	6.75	8.22	9.40	8.83	4.41	4.58	4.13	12.14	11.66	12.49
XIV.	7.01	7.43	6.61	9.10	8.49	8.13	4.06	4.36	4.21	12.36	11.04	11.49
XV.	7.06	7.25	6.44	6.80	7.21	7.92	4.39	4.09	4.17	11.19	11.86	10.37
XVI.	6.88	6.36	6.52	7.22	7.39	7.83	4.52	4.16	3.94	12.45	11.64	11.21

TABLE V-A.  
MILLIONS OF BACTERIA PER GRAM OF AIR-DRY SOIL.

Media.	Soil No. 9			Soil No. 10			Soil No. 11			Soil No. 12		
I.	6.61	6.06	6.44	4.12	4.58	4.33	4.91	4.95	4.87	7.80	6.39*	7.49
II.	7.48	7.29	7.71	4.41	4.11	4.08	6.13	6.30	6.27	9.68	9.77	9.81
VI.	6.87	7.06	7.39	4.01	4.36	4.44	5.70	6.04	5.83	9.21	9.08	9.59
XII.	6.38	6.86	6.52	3.99	4.13	4.22	4.87	5.13	5.34	8.33	8.40	8.11
XIII.	7.29	7.52	7.36	4.43	4.87	4.27	6.21	6.31	5.88	9.78	9.41	9.82

\* Omitted from average.

TABLE VI.  
MILLIONS OF BACTERIA PER GRAM OF AIR-DRY SOIL.

Media.	Soil No. 13			Soil No. 14			Soil No. 15			Soil No. 16		
II.	14.80	16.30	16.80	7.35	7.00	7.70	3.99	3.20	3.46	9.99	9.25	10.40
XIII.	9.80*	15.30	15.70	8.05	6.90	8.25	2.46	2.73	2.86	9.17	9.50	8.18
XVII.	14.60	13.80	Lost	7.75	9.60	7.96	3.00	3.12	2.86	7.04	7.50	7.86
XVIII.	16.40	18.20	Lost	7.90	8.20	10.30	3.86	3.99	*2.40	9.71	10.70	10.81
XIX.	14.80	16.60	14.36	7.10	7.70	Lost	2.60	2.50	Lost	9.37	9.64	7.83

\* Omitted from average.

TABLE VII.  
MILLIONS OF BACTERIA PER GRAM OF AIR-DRY SOIL.

Media.	Soil No. 17			Soil No. 18			Soil No. 19			Soil No. 20		
II.	14.70	12.70	13.30	6.41	6.82	6.22	2.30	1.94	2.40	5.40	5.35	4.33
XVIII.	15.65	17.10	15.30	6.96	7.01	7.34	2.61	2.18	2.46	5.60	4.47	4.61
XX.	15.60	14.30	14.95	7.10	6.78	7.25	3.46	2.93	3.07	4.67	4.54	5.74





# THE INFLUENCE OF VARIOUS SALTS ON THE GROWTH OF SOYBEANS.\*

BY JOHN W. SHIVE,

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An experiment conducted by Dr. B. H. A. Groth, the object of which was to test the effect of various salts on the growth of soybeans (*Glycine hispida*) and prairie berries (*Solanum nigrum*), was terminated in May, 1915. During the following summer this experiment was repeated in part, the same pots of soil being again employed without alteration. In preparation for the repeated experiment, the soil in each pot was thoroughly mixed and a sufficient quantity of tap water added to each culture to restore approximately its original water content (11.1+per cent on the air-dry basis). The series of pots from which a crop of soybeans had been harvested were again planted with soybeans. The series of cultures which had yielded a crop of prairie berries were again planted with prairie berry seeds. The latter, however, failed to germinate and this series of cultures was discontinued.

The soil used in the original experiment and also employed in the repeated experiment, consisted of a mixture of equal parts by weight of air-dry, white, sea-shore sand and rich garden soil. Each pot contained 4.5 kg. of this mixture.

In the original experiment, the salts added to the soil-sand mixture comprised the carbonates, chlorides, nitrates, phosphates, and sulphates of sodium, potassium, calcium, and ammonium, each used singly. The soluble salts were added to the soil in the form of solutions. The amount of salt in question required for a culture was dissolved in 500 c.c. of water and this solution was then added to 4.5 kg. of the soil and thoroughly mixed with it. The difficultly soluble salts were added to the soil in the powdered form. To 4.5 kg. of the soil was added the required amount of powdered salt in question and to the whole was then added 500 c.c. of water with thorough mixing.

Each salt was employed at five different concentrations, each of which represents a definite percentage value for all the salt radicals (theoretical atomic groups),  $\text{Na}_2\text{O}$ ,  $\text{K}_2\text{O}$ ,  $\text{CaO}$ , and  $\text{NH}_4$  for the carbonates, and  $\text{NO}_3$ ,  $\text{PO}_4$ ,  $\text{CO}_3$  and  $\text{Cl}$  for the nitrates, phosphates, sulphates, and chlorides, respectively. These five different concentrations of the salt radicals are 0.05, 0.10, 0.15, 0.20, and 0.30 per cent of the weight of the air-dry soil (4.5 kg. to each culture).

The cultures of this experiment may be divided into four groups with reference to the class of salts employed, the cultures containing

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the sodium, potassium, calcium, and ammonium salts, respectively, comprising the four groups. Each group of cultures employed five different salts (carbonates, chlorides, nitrates, phosphates, and sulphates) at five different concentrations, making a total of twenty-five cultures in each group. In addition each group contained a control culture consisting of the same soil-sand mixture as the other cultures, but containing no salts.

Table I gives the chemical formulae of the salts employed in each group of cultures, also the radicals upon which concentration calculations are based, and the actual weight in grams of the salt in each culture required to produce the various concentrations of the radicals in question.

TABLE I.

ACTUAL WEIGHT IN GRAMS OF THE SALTS EMPLOYED IN EACH CULTURE, CALCULATED FROM THE PER CENT VALUES OF THE SALT RADICAL CONCENTRATIONS.

Chemical formulae of salts	Salt radicals	Weight of salts required to produce salt radical concentrations of				
		0.05%	0.10%	0.15%	0.20%	0.30%
		gm.	gm.	gm.	gm.	gm.
$\text{Na}_2\text{CO}_3$	$\text{Na}_2\text{O}$	4.518	9.035	13.553	18.070	27.105
$\text{NaCl}$	$\text{Cl}$	3.710	7.420	11.130	14.840	22.260
$\text{NaNO}_3$	$\text{NO}_3$	3.083	6.165	9.248	12.330	18.495
$\text{Na}_3\text{PO}_4$	$\text{PO}_4$	9.005	18.009	27.014	36.018	54.027
$\text{Na}_2\text{SO}_4$	$\text{SO}_3$	3.950	7.900	11.850	15.800	23.700
$\text{K}_2\text{CO}_3$	$\text{K}_2\text{O}$	3.310	6.620	9.930	13.241	19.860
$\text{KCl}$	$\text{Cl}$	4.733	9.465	14.198	18.930	28.395
$\text{KNO}_3$	$\text{NO}_3$	3.670	7.340	11.010	14.680	22.020
$\text{K}_3\text{PO}_4$	$\text{PO}_4$	5.023	10.045	15.068	20.090	30.135
$\text{K}_2\text{SO}_4$	$\text{SO}_3$	4.845	9.690	14.535	19.380	29.070
$\text{CaCO}_3$	$\text{CaO}$	4.020	8.040	12.060	16.080	24.120
$\text{CaCl}_2$	$\text{Cl}$	3.525	7.050	10.575	14.100	21.150
$\text{Ca}(\text{NO}_3)_2$	$\text{NO}_3$	4.288	8.573	12.863	17.150	25.725
$\text{Ca}_3(\text{PO}_4)_2$	$\text{PO}_4$	3.666	7.332	10.998	14.664	21.996
$\text{CaSO}_4$	$\text{SO}_3$	4.838	9.675	14.513	19.350	29.025
$(\text{NH}_4)_2\text{CO}_3$	$\text{NH}_4$	6.185	12.370	18.555	24.740	37.110
$\text{NH}_4\text{Cl}$	$\text{Cl}$	3.393	6.785	10.178	13.570	20.355
$\text{NH}_4\text{NO}_3$	$\text{NO}_3$	2.905	5.810	8.715	11.620	17.430
$(\text{NH}_4)_2\text{HPO}_4$	$\text{PO}_4$	3.125	6.250	9.375	12.500	18.750
$(\text{NH}_4)_2\text{SO}_4$	$\text{SO}_3$	3.710	7.420	11.130	14.840	22.260

The seed used in this experiment was raised on the experiment plot of the botanical department of this station, and under favorable conditions yielded 96 per cent strong germination. The seeds were planted directly in the soil, ten seeds to each pot, at a depth of from 2 to 3 cm. Only five plants, however, were allowed to grow in each culture. In the higher concentrations the seeds in a number of cases failed to germinate and such cultures were discontinued.

The cultures were conducted in the experiment greenhouse during the time period from August 3 to September 20, 1915. The water lost

from each culture by transpiration and by evaporation from the soil surface was restored every second day by the method of weighing. The water added to the culture was, in each case, poured through a test tube open at both ends and placed vertically in the soil so as to extend about half way to the bottom of the pot. This prevented flooding of the surface of the soil. At the end of the time period of 48 days the plants were harvested. The tops were severed from the roots at the surface of the soil, placed in weighing bottles and dried for two days at a temperature of about 96° C. and from four to five hours longer at a temperature of from 102° C. to 104° C. The dry weights of the tops were then obtained in the usual way.

The numerical data of the yields of tops are presented in Table II. The dry weights of tops are given in this table relative to the average dry weights of tops of the control cultures taken as 1.00. The actual average dry weight, in grams, of these controls is given in parentheses in the table heading for the dry weight columns. The actual weight of any culture may be obtained by multiplying its relative weight by the actual weight of the average control culture. The blank spaces in the table indicate either failure of the seeds to germinate or failure of the seedlings to develop after germination had taken place.

TABLE II.  
RELATIVE DRY WEIGHT OF SOYBEAN TOPS GROWN 48 DAYS IN SOIL SAND MIXTURE WITH FIVE DIFFERENT CONCENTRATIONS OF THE SALT RADICALS.

Chemical formulae of salts	Salt radicals	Dry weight of tops relative to the average dry weight of the controls (5.48 grams) taken as 1.00				
		0.05% concentration	0.10% concentration	0.15% concentration	0.20% concentration	0.30% concentration
Na <sub>2</sub> CO <sub>3</sub>	Na <sub>2</sub> O	1.02	.93	....	....	....
NaCl	Cl	.49	.50	....	....	....
NaNO <sub>3</sub>	NO <sub>3</sub>	.84	.82	.98	....	....
Na <sub>2</sub> PO <sub>4</sub>	PO <sub>4</sub>	.63	.64	....	....	....
Na <sub>2</sub> SO <sub>4</sub>	SO <sub>3</sub>	.51	.45	.73	.52	....
K <sub>2</sub> CO <sub>3</sub>	K <sub>2</sub> O	.98	.91	.47	....	....
KCl	Cl	.64	.55	....	....	....
KNO <sub>3</sub>	NO <sub>3</sub>	.80	.78	....	....	....
K <sub>2</sub> PO <sub>4</sub>	PO <sub>4</sub>	.74	.52	....	....	....
K <sub>2</sub> SO <sub>4</sub>	SO <sub>3</sub>	.54	.54	.62	.58	....
CaCO <sub>3</sub>	CaO	1.28	1.33	1.35	1.20	.80
CaCl <sub>2</sub>	Cl	.60	.57	....	....	....
Ca(NO <sub>3</sub> ) <sub>2</sub>	NO <sub>3</sub>	.73	.78	.69	.97	....
Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	PO <sub>4</sub>	.67	.62	.35	.30	.35
CaSO <sub>4</sub>	SO <sub>3</sub>	.56	.42	.68	.57	.32
(NH <sub>4</sub> ) <sub>2</sub> CO <sub>3</sub>	NH <sub>4</sub>	.76	.72	.44	.65	....
NH <sub>4</sub> Cl	Cl	.43	.34	....	....	....
NH <sub>4</sub> NO <sub>3</sub>	NO <sub>3</sub>	.54	.43	....	....	....
(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	PO <sub>4</sub>	.51	.43	.33	.49	....
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	SO <sub>3</sub>	.62	.39	.41	....	....

From Table II it will be observed that only with 0.05 and 0.10 per cent concentrations did germination and development take place in all the cultures. With 0.15, 0.20, and 0.30 per cent concentrations the number of cultures which failed were 9, 12, and 17, respectively, out of a possible total of twenty cultures for each concentration. On the one hand, the failure in the germination or development of these cultures is undoubtedly related to the concentration of the salts in the soil solution, which in the high concentrations here employed, act osmotically to offer resistance to water entrance into the seeds and young roots, and, as might be expected, the number of failures increased as the concentration increased. On the other hand, the toxic action of the salts in each of these cultures may be a factor in the prevention or retardation of development. The former is an effect of the physical properties of the soil solution; the latter an effect of its chemical properties. It is, of course, entirely possible, and indeed probable, that these two factors acting at the same time are responsible for the results noted. To what extent failure or retardation in development of these cultures is due to one or the other of these two factors has not been determined.

Further inspection of Table II brings out the fact that only five cultures produced yields of tops superior to the average yield of the four control cultures. One each of these occurred with the 0.05, 0.10, 0.15, and 0.20 per cent concentrations of  $\text{CaO}$ , and the remaining one with the 0.05 per cent concentration of  $\text{Na}_2\text{O}$ . All other cultures produced yields inferior to the average yield of the control cultures. This retardation in the growth of the plants must be regarded as directly related to the unfavorable influence of the salts employed in these cultures, either by a toxic action affecting the life processes of the plants in a chemical way, or by giving rise to osmotic activities in the soil solutions resulting in too great resistance to water entrance into the roots in quantities adequate to supply the loss by transpiration and that used in the metabolic processes of the plant.

The relative toxic influences of the salts upon the growth of soybeans may be studied from the standpoint of relative dry weights as a criterion. With this point in view the dry weights of the tops, relative to the average control, of the plants grown in the cultures containing the sodium salts at the 0.05 per cent concentration were arranged in the order of their magnitudes, beginning with the highest. These form a rather uniformly decreasing series of numbers which were next plotted to form a graph shown as the heavy black line in Figure 1 (lower group of graphs). Here the abscissas were chosen arbitrarily to represent the different salts of the same base, the acid radicals of which are placed below. These acid radicals are the same for each group of salts. The

ordinates represent the relative dry weight values. With the same abscissas the corresponding dry weight values for the three remaining groups of cultures (potassium, calcium, and ammonium salts), at the 0.05 per cent concentration, were plotted, using the same scale for the ordinates, thus forming four graphs, each graph representing a single group of five cultures. The relative dry weight values for the four groups of cultures, all at the 0.10 per cent concentration, were plotted in a similar manner on the same sheet, using the same abscissas and the same ordinates. Curves of the dry weight values for the higher concentrations are not here presented since none of the groups is complete.

From Figure 1 it is at once clear that at the 0.05 and 0.10 per cent concentrations all the carbonates agree in showing higher dry weight yields than do any of the other salts in their respective groups. The dry weight values, arranged in the order of their magnitudes from the highest to the lowest yields, occur with the carbonates, nitrates, phosphates, sulphates, and chlorides respectively. At the 0.05 per cent concentration, however, ammonium sulphate produced a higher yield of tops than did the corresponding phosphate, and both calcium and potassium chlorides yielded higher dry weights of tops than did the corresponding sulphates. At the 0.10 per cent concentration, potassium sulphate yielded a slightly higher dry weight value than did the corresponding phosphate, and the chlorides of sodium, potassium, and calcium produced somewhat higher yields than did the corresponding sulphates, potassium chloride yielding also a slightly higher dry weight value than did the corresponding phosphate. The yields from cultures containing ammonium salts, in every instance are lower in value than the yields from the corresponding cultures of each of the other groups, excepting the yield from the culture containing ammonium sulphate at the 0.05 per cent concentration already noted.

With the 0.05 and 0.10 per cent concentrations, the cultures containing calcium carbonate show a markedly higher dry weight yield than does the average control. The culture containing sodium carbonate with the 0.05 per cent concentration also shows a slight improvement over the average control. The remaining cultures containing carbonates show yields somewhat lower than the average yields from the controls, and this becomes marked in the case of the cultures containing ammonium carbonate. The dry weight yields of all the other cultures with the 0.05 and 0.10 per cent concentrations here considered, fall far below the average dry weight of the controls. The relative differences in the degree of the toxic influence of the various salts employed, manifesting itself in retarded growth, are indicated by the gradual downward slope of all the graphs in Figure 1. It will be observed that the ammonium salts, judging from the criterion of dry weight yields, exert a markedly

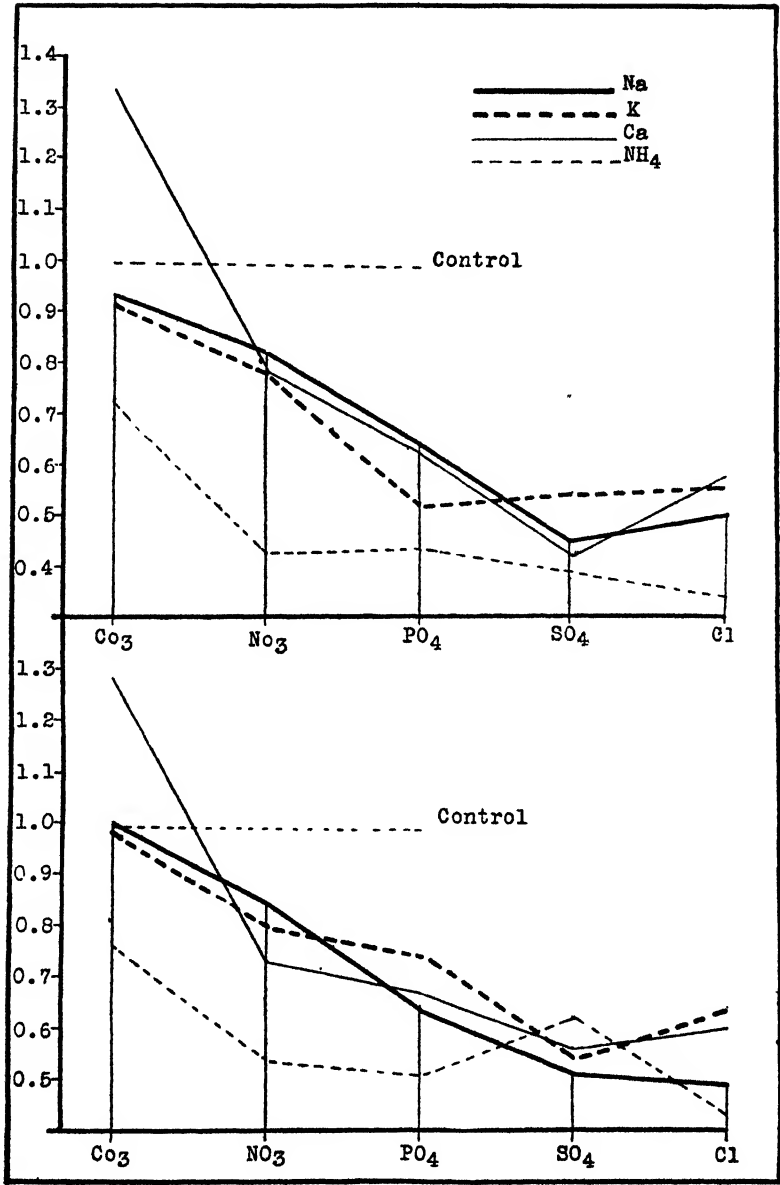


Figure 1—Graphs showing the relative dry weight values of soybeans grown in various cultures containing different salts.

greater toxic influence upon the growth of soybeans than do any of the corresponding salts of sodium, potassium, or calcium. There is, however, one notable exception in the case of ammonium sulphate with the 0.05 per cent concentration. Here the toxic influence, as indicated by the dry weight values, is somewhat less than that of the corresponding salts of the other basic elements (sodium, potassium, and calcium). With the 0.10 per cent concentration, however, this condition is reversed.

No marked differences excepting those of size occurred in the tops of the soybeans during the first twelve days of growth. At the end of this time, however, evidences of disturbed growth began to appear in certain cultures, first in the calcium phosphate cultures and later in all the phosphate cultures excepting those with the 0.05 per cent concentration. This disturbance manifested itself first in injury to the cotyledons. It consisted of a reddish-brown discoloration around the margins of these organs, gradually spreading toward the center. In severe cases the entire cotyledon became discolored and the death of the organ quickly ensued. In case of slight injury to the cotyledons, in addition to the marginal discoloration, reddish-brown spots also appeared at irregular intervals over the surface. In many cases the cotyledons completely recovered from this form of the injury, continuing normal during the remainder of the growth period. Usually an interval of several days elapsed between the time when the injury first manifested itself on the cotyledons and its appearance on the first pair of foliage leaves. The injury spread, in severe cases, to include all the leaves of the plant. In the leaves the disturbance appeared first as small, yellowish, translucent spots, which quickly took on the characteristic reddish-brown hue. These spots first appeared near the margin of the leaf and gradually increased in size and spread to cover the entire leaf, when death and falling of the leaf quickly followed. A foliage leaf once injured never recovered, however slight the injury may have been.

This injury occurred only with the cultures containing the phosphates and was most severe in the calcium phosphate cultures, all of which were dead at the time of harvesting. With the sodium, potassium, and ammonium cultures no injury occurred at the 0.05 per cent concentration, although at the 0.10 per cent concentration all the plants were severely injured, and with the higher concentrations all the sodium and potassium phosphate cultures failed, while with 0.15 and 0.20 per cent concentrations of ammonium phosphate the plants were dead at the time of harvesting.

The injury here described seems to be related directly to the phosphate salts. However, not sufficient data are at hand at the present time to warrant any definite conclusions. The reaction of soybean plants to



ward the phosphate salts here dealt with singly and in combination with other salts, is at the present time the subject of further investigation.

Further evidences of disturbed growth appeared in the plants grown in the soil-sand mixture containing the ammonium salts. These plants as a whole were characterized by an unusually dark green coloration of the leaves, which may have been the result of an abundant supply of nitrogen, but here, at least, it is by no means an indication of a healthful condition. Not only had the plants grown in the lowest concentration (0.05 per cent) of the ammonium salts a decidedly deeper green coloration than had the plants from cultures containing the salts of sodium, potassium, or calcium, but the intensity of this coloration was correspondingly more pronounced as the concentration of the salts in the cultures was greater, and the dry weight yields correspondingly less. The plants from cultures containing ammonium salts were further characterized by exceedingly short leaf petioles, which gave the plants a stunted appearance.

The present experiments, as well as similar experiments immediately preceding this, have yielded little that might be construed as conclusive, with respect to the toxic or beneficial influences of the salts here employed, upon plant reactions. Nevertheless, these experiments have been a fruitful source of suggestions for constructive investigation.

# FACTORS INFLUENCING THE PROTEIN CONTENT OF SOYBEANS,\*

BY JACOB G. LIPMAN, *Director*, and A. W. BLAIR, *Associate Soil Chemist*,  
of the New Jersey Agricultural Experiment Stations.†

This is a continuation of work that was begun in the summer of 1914, an account of which was given in the Annual Report of the Experiment Station for that year.‡ It was there pointed out that the protein content of soybeans may be considerably influenced by such factors as thickness of planting and date of harvesting, and also that the different varieties show considerable variation in protein content. Different fertilizing materials did not appear to make any material change in the protein content.

## SERIES I.

### *Rate of Seeding.*

The soil for this experiment was a Collington sand, slightly alkaline in reaction. Glazed earthenware pots, holding 20 pounds of soil each, were used. To the soil for each pot there was added 4 gm. of acid phosphate, and 2 gm. muriate of potash, in order that there should be no deficiency of minerals. On May 13th the pots were seeded to Swan soybeans, all being inoculated with an infusion made from soil in which soybeans had been grown. The beans were allowed to grow until the pods were well set, but before the leaves began to fall to any extent, they were harvested as forage.

The method of carrying out the experiment and the yield of dry matter and nitrogen are shown in Table I. With slight exception the increased rate of seeding gave increased returns in yield of dry matter, the thicker plantings giving about double the yield given with 2 to 8 plants per pot.

The percentage of nitrogen in the dry matter is slightly higher with the small number of plants to a pot than with the larger numbers. This is in accord with last year's results, though the differences for this year are somewhat more pronounced. In the matter of the total nitrogen recovered, the pots with 14 to 30 plants stand far ahead of those with 2 to 8 plants. This is due largely to the greater yield of dry matter of the former. With 20 to 30 plants a pot the yield of nitrogen is more than twice that with 2 to 4 plants a pot. As intimated in the previous report,

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† The field work in connection with the experiments here recorded was under the direction of Mr. L. K. Wilkins and the analyses were made by Mr. H. C. McLean, of the New Jersey Agricultural Experiment Station.

‡ New Jersey Agricultural Experiment Station, Annual Report, 1914, p. 240-245.

this would seem to indicate increased or intensified utilization of atmospheric nitrogen by means of symbiotic bacteria, with the thicker plantings. Certainly much more nitrogen is recovered from a given area, and if, as is generally conceded, much of this nitrogen is drawn from the atmosphere, there is good reason for thick seeding of soybeans.

TABLE I.  
SOYBEANS—RATE OF SEEDING.

No.	Plants Per Pot	Dry Matter gm.		Per cent Nitrogen	Total Nitrogen mg.	
		Per Pot	Average		Per Pot	Average
1		24.5		3.440	843	
2	4 thinned to 2....	27.8	26.15	3.321	924	884
3		19.5		3.264	636	
4	6 thinned to 4....	26.2	22.85	3.272	858	747
5		39.8		3.272	1302	
6	12 thinned to 8....	29.5	34.65	2.690	793	1048
7		41.0		2.986	1224	
8	18 thinned to 14....	50.8	45.90	3.253	1653	1439
9		58.8		2.995	1760	
10	24 thinned to 20....	56.5	57.65	3.035	1715	1738
11		53.7		2.848	1529	
12	30 thinned to 25....	62.5	58.10	3.006	1880	1705
13		63.2		2.986	1877	
14	36 thinned to 30....	63.4	63.30	2.867	1816	1847

#### SERIES II.

##### *Can nodule formation be depressed?*

It is generally believed that with an abundant supply of available nitrogen in the soil, leguminous crops do not draw as much of their nitrogen from the air as they do with an insufficient supply of soil nitrogen. With the hope of throwing further light on this question it was decided to try the effect of gradually increasing amounts of nitrogenous fertilizers in the growing of soybeans, to determine whether or not nodule formation may in this way be depressed. The experiment was carried out in glazed earthenware pots which held 20 pounds of white sand. To each pot the following were added: 4 gm. acid phosphate, 2 gm. potassium chloride, 10 gm. ground limestone, 0.5 gm. magnesium sulphate, and 0.25 gm. ferric sulphate. The pots were planted to Guelph soybeans on May 21st. The pots were inoculated by sprinkling with an infusion made with soil taken from a field where soybeans had grown successfully. The beans were allowed to grow until the pods were well filled. They then were harvested, roots and tops, the nodules counted, and the tops and roots weighed and prepared for analysis. Table II shows the plan for the special treatment and the weights of dry matter and nitrogen recov-

ered in both tops and roots. The amounts of ammonium sulphate and dried blood used are equivalent to 0.5 gm., 1 gm., 2 gm., and 4 gm. of nitrate of soda (15.93 per cent nitrogen), so that the results are comparable so far as the amount of nitrogen applied is concerned. Referring first to the part of the table dealing with the tops, it will be noted that, with slight exception, the applications of nitrogen have resulted in some increase in yield of dry matter, over the checks. With the dried blood there is a gradual increase in dry matter as the amount of blood applied is increased. The percentage of nitrogen in the dry matter is irregular and does not appear to be influenced greatly one way or the other.

TABLE II.  
SOYBEAN VINES—CAN NODULE FORMATION BE DEPRESSED?

No.	Special Treatment	Dry Matter gm.		Per Cent Nitrogen	Total Nitrogen mg.		Increase over Check mg.
		Per Pot	Average		Per Pot	Average	
1		26.3		2.816	740		
2	No Nitrogen .....	20.5	23.40	3.241	664	702*	...
3		21.0		2.923	614		
4	.5 gm. Nitrate of Soda.	20.4	20.70	3.004	612	613	...
5		37.9		3.241	1229		
6	1 gm. Nitrate of Soda.	41.4	39.65	3.093	1281	1255	525
7		26.0		3.172	824		
8	2 gm. Nitrate of Soda.	39.0	32.50	3.153	1230	1027	297
9		30.6		3.104	950		
10	4 gm. Nitrate of Soda.	36.7	33.65	2.974	1091	1021	291
11		22.0		3.262	717		
12	No Nitrogen .....	24.0	23.00	3.162	759	738*	...
13		22.0		3.341	735		
14	.378 gm. Sul. Amm'a.	29.2	25.60	3.151	920	828	98
15		28.0		3.322	930		
16	.756 gm. Sul. Amm'a.	29.2	28.60	3.172	926	928	198
17		30.2		3.122	943		
18	1.512 gm. Sul. Amm'a.	37.0	33.60	3.151	1166	1055	325
19		26.5		2.955	782		
20	3.024 gm. Sul. Amm'a.	32.8	29.65	3.271	1074	928	198
21		18.2		3.182	579		
22	No Nitrogen .....	28.2	23.20	3.271	922	751*	...
23		25.5		3.202	817		
24	.6848 gm. Dried Bl'd.	24.0	24.75	3.202	768	793	63
25		26.5		3.103	822		
26	1.3697 gm. Dried Bl'd.	28.2	27.35	3.331	939	881	151
27		26.0		3.024	786		
28	2.7394 gm. Dried Bl'd.	30.2	28.10	3.222	972	879	149
29		29.0		2.658	771		
30	5.4788 gm. Dried Bl'd.	35.0	32.00	3.240	1134	952	222

What has been said with reference to the tops is generally true of the roots also. The percentage of nitrogen in the dry matter of the roots is irregular and not in proportion to the amount of nitrogen applied. The same is true with reference to the number of nodules.

\* Averaged for check.

It therefore appears that, in sand culture at least, nodule formation is not depressed by applications of nitrogenous fertilizers. Evidently the plants did use some of the applied nitrogen, as evidenced by the gradual increase, in some cases, in the amount of nitrogen recovered, as the amount of nitrogen applied was increased. The excellent growth made by the checks, however, would lead to the belief that a large part of the nitrogen recovered, perhaps two-thirds or three-fourths, was secured from the atmosphere.

TABLE III.  
SOYBEAN ROOTS—CAN NODULE FORMATION BE DEPRESSED?

No.	Special Treatment			Dry Matter gm.		Per Ct. Nit'gen	Total Nitrogen mg.		Inc. over Check mg.
		No. of Nodules		PerPot	Ave'ge		Per Pot	Ave'ge	
		Per Pot	Ave'ge						
1		275		7.8		.600	46.8		(check)
2	No Nitrogen . . . . .	290	28.2	3.3	5.55	.974	32.2	39.50*	39.90
3		243		6.8		.817	55.6		
4	.5 gm. Nitrate of Soda.	156	200	6.7	6.75	.699	46.8	51.20	11.30
5		331		7.6		.699	53.1		
6	1 gm. Nitrate of Soda.	321	326	9.1	8.35	.718	65.4	59.25	19.35
7		242		8.0		.551	44.1		
8	2 gm. Nitrate of Soda.	222	232	8.0	8.00	.688	55.0	49.55	9.65
9		142		6.8		.895	60.8		
10	4 gm. Nitrate of Soda.	199	170	8.0	7.40	.777	62.2	61.50	21.60
11		176		5.5		.826	45.4		(check)
12	No Nitrogen . . . . .	309	242	7.1	6.30	.502	35.6	40.50*	39.90
13		158		5.8		.797	46.2		
14	.378 gm. Sul. Amm'a.	231	194	6.3	6.05	.669	42.2	44.20	4.30
15		253		6.1		.748	45.6		
16	.756 gm. Sul. Amm'a.	248	250	7.6	6.85	.590	44.8	45.20	5.30
17		238		7.7		.876	67.4		
18	1.512 gm. Sul. Amm'a.	432	335	8.2	7.95	.826	67.8	67.60	27.70
19		262		7.1		1.082	76.8		
20	3.024 gm. Sul. Amm'a.	250	256	10.5	8.80	.728	76.4	76.60	36.70
21		185		3.9		.974	38.0		(check)
22	No Nitrogen . . . . .	253	219	9.1	6.50	.453	41.4	39.70*	39.90
23		223		5.8		.836	48.5		
24	.6848 gm. Dried Bl'd.	293	258	7.0	6.40	.748	52.4	50.45	10.55
25		205		7.7		.876	67.4		
26	1.3697 gm. Dried Bl'd.	318	261	6.5	7.10	.925	60.1	63.75	23.85
27		206		7.1		.708	50.3		
28	2.7394 gm. Dried Bl'd.	235	220	7.5	7.30	.826	62.0	56.15	16.25
29		179		3.6		.944	34.0		
30	5.4788 gm. Dried Bl'd.	381	280	8.1	5.85	.728	58.9	46.45	6.55

\* Averaged for check.

### SERIES III.

#### Varieties.

The soil used for this experiment was a loamy silt originally acid in reaction. The acidity was corrected by the use of a liberal application of ground limestone. No other fertilizing materials were used. The glazed earthenware pots used held 8 pounds of this soil. On May 29th the pots

were seeded, in duplicate, to fifteen different varieties of soybeans. All pots were inoculated with an infusion made from a soil taken from a plot on which soybeans had previously been grown. The varieties used and the yields of dry matter and nitrogen are indicated in Table IV.

TABLE IV.  
VARIETIES OF SOYBEANS.

Number	Variety	Green vines and pods			Matured							
		Dry Matter gm.	% Nitrogen	Total Nit'g'n mg.	Number	Stems			Seeds			
						Dry Matter gm.	% Nitrogen	Nit. in stems mg.	Dry Matter gm.	% Nitrogen	Nit. in seeds mg.	Total Nit'g'n mg.
1	Guelph	24.2	3.046	737	2	10.5	.885	92.9	10.0	6.160	616.0	708.9
3	Ohio 9035	24.5	2.680	656	4	9.0	1.063	95.7	4.1	6.188	253.7	349.4
5	Swan	26.2	2.839	744	6	10.2	.935	95.4	6.7	6.533	437.7	533.1
7	Ebony	26.2	3.460	906	8	9.3	1.515	140.9	7.0	6.612	462.8	603.7
9	Tarheel	42.0	2.803	1177	10	16.0	.974	155.8	14.9	6.198	923.5	1079.3
11	Edna	24.6	2.978	732	12	6.9	1.181	81.5	7.0	5.925	414.8	496.3
13	Ito San	22.5	3.026	681	14	7.0	1.210	84.7	6.8	6.385	434.2	518.9
15	Black eyebrow	22.4	2.769	620	16	7.5	.767	57.5	9.0	6.660	599.4	656.9
17	Hollybrook	26.4	2.996	791	18	8.0	.826	66.1	9.0	6.775	609.8	675.9
19	Wilson	21.7	2.670	579	20	8.0	1.279	102.3	3.6	5.372	193.4	295.7
21	Manhattan	21.6	3.153	776	22	8.0	.679	54.3	9.8	6.809	667.3	721.6
23	Claud	23.0	2.871	660	24	9.3	1.092	101.6	8.1	5.205	421.6	523.2
25	Medium Yellow	29.8	2.512	749	26	9.2	1.004	92.4	7.0	5.952	416.6	509.0
27	Manchu	20.0	3.056	611	28	6.5	1.013	65.8	8.5	6.425	546.1	611.9
29	Baird	27.2	2.678	728	30	9.0	1.053	94.8	8.8	6.169	542.9	637.7

It should be pointed out that half the pots—the odd numbers—were harvested as forage, while the even numbers were left to ripen seed, and were harvested as seed and stalks separately. The leaves had largely disappeared from the stalks by the time the pods had ripened.

Of those harvested as forage nine varieties, Tarheel, Medium Yellow, Baird, Hollybrook, Ebony, Swan, Manhattan, Edna, and Guelph, in the order named, yielded more than 24 gm. of dry matter. All of these varieties also yielded more than 700 mg. of nitrogen. Tarheel gave the highest yields of dry matter and nitrogen, while Ebony stands second in the yield of nitrogen, and Medium Yellow second in the yield of dry matter. Six varieties, Ebony, Manhattan, Manchu, Guelph, Ito San, and Hollybrook, show 3 per cent or over of nitrogen in the dry matter. Four of these, Ebony, Guelph, Manhattan, and Hollybrook, stood considerably above 3 per cent in the 1914 test. Manchu and Ito San were not included in the experiment of last year. Of those harvested after seeds had ripened, Tarheel, Guelph and Swan lead in the yield of dry stalks, while Tarheel, Guelph and Manhattan lead in the production of seed. As might be expected, the percentage of nitrogen in the dry stalks is decidedly less than in the vines as harvested for forage. This is no doubt largely accounted for by the separation of the beans and by the loss of the leaves.

Five varieties, Manhattan, Hollybrook, Black Eyebrow, Ebony and Swan, show more than 6.5 per cent of nitrogen in the dry beans, and six varieties, Manchou, Ito San, Tarheel, Ohio 9035, Baird and Guelph, show between 6 and 6.5 per cent of nitrogen. Nearly all of these are likewise among those that yielded the highest percentage of nitrogen and the highest total nitrogen in the forage.

A word of caution should be given in regard to the Tarheel, which has shown up so well in this experiment. It is a late, slow-growing bean, and unless planted early and in good soil it would probably not mature seed. It makes a large growth of vines and if planted early would be good as a forage crop or as a green manure. Otherwise it could not be recommended for central or northern New Jersey.

From the above it will be noted that the varieties here tested differ considerably both as to yield of dry forage and beans, and also as to percentage of nitrogen in the dry matter. For example, the lowest percentage of nitrogen in the dry beans is 5.21 and the highest 6.81, a difference of 1.6 per cent, which is equivalent to 10 per cent of protein.

The work should be repeated in duplicate, however, before definite conclusions on this point are arrived at.

Certain varieties, as Guelph, Ohio 9035, Swan, Ebony, Tarheel (with the exceptions already noted), Hollybrook and Manhattan, seem to stand out prominently as giving a good yield of dry matter and a high percentage of nitrogen, and whether one is selecting the beans for a feeding material or for a green manure crop, this is important. In the case of a green manure crop, one of the high yielding varieties with a high nitrogen content will add to the soil far more nitrogen per acre than one that is low in both. In a field test in 1914\* five varieties grown on limed plots gave an average of 6.7 per cent of nitrogen in the shelled beans, while the same varieties grown on unlimed plots gave an average of 6.2 per cent of nitrogen in the shelled beans.

#### VARIETY TEST OF SOYBEANS—FIELD EXPERIMENT.

Continuing the work of 1913 and 1914, an account of which was given in Bulletin No. 282 of this Station, fifteen varieties of soybeans were grown again this year on field plots varying in size from  $1/80$  to  $1/20$  of an acre.

All plots had previously been inoculated, and as in previous years acid phosphate was applied at the rate of 400 pounds and muriate of potash 100 (formerly 200) pounds per acre. In the spring of 1913 certain of the plots received a treatment of ground limestone at the rate of 2 tons per acre, while certain other plots did not receive the lime treatment. The soil is a loam to sandy loam, on which leguminous crops have been grown for some years.

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\* Factors Influencing the Protein Content of Soybeans. New Jersey Agricultural Experiment Station, Bulletin No. 282, p. 13.

CROP OF 1915.

The ground was broken and prepared about the last of April, and about the middle of May the fertilizers were applied and the different varieties planted in rows 33 inches apart. The germination was fair and the beans were cultivated during the season in the usual way.

The beans were harvested by pulling up the vines after the pods had matured and standing in cone shaped bunches (roots up) to dry out. By this method of harvesting the leaves are largely left on the field. When they were sufficiently dry they were weighed, stalks and pods together, threshed, and the shelled beans weighed and ground for analysis. The weights, calculated to the acre basis, and the percentage of nitrogen in the shelled beans are shown in Table V.

TABLE V.  
YIELD OF DRY MATTER, AND NITROGEN CONTENT OF SOYBEANS, 1915.  
(Calculated to acre basis.)

Variety	Dry Weight Vines and Pods, lbs.		Dry Weight Shelled Beans, lbs.		Per Cent Nitrogen Shelled Beans	
	Limed	Unlimed	Limed	Unlimed	Limed	Unlimed
Baird .....	....	....	...	...	6.868	....
Black Eyebrow .....	2240	1360	680	376	6.480	6.129
Claud .....	3680	....	1064	...	5.887	....
Ebony .....	2400	....	944	...	6.743	....
Edna .....	2480	....	1240	...	6.354	....
Guelph .....	1600	....	536	...	6.497	....
Hollybrook .....	2280	1920	756	672	6.868	6.148
Ito San Plot 67 .....	2400	..	860	...	6.547	....
Manchu .....	2220	1380	1044	600	6.497	5.682
Manhattan .....	2600	....	748	...	5.916	....
Medium Yellow .....	2960	1680	1088	792	6.264	5.720
Ohio 9035 .....	2960	2080	1136	592	5.897	5.712
Swan .....	3480	1320	1278	564	6.663	5.964
Tarheel .....	4800	2240	616	272	6.178	6.062
Wilson .....	1800	....	984	...	5.923	....
Average .....	2991*	1711	943*	553	6.407*	5.917

\* The average for those varieties only that have corresponding unlimed plots.

For the limed sections Tarheel gave a yield of 4800 pounds of total dry matter, followed in order by Claud with a yield of 3680 pounds, and Swan with 3480 pounds. All other varieties fell below 3000 pounds of total dry matter. The highest yield of total dry matter on the unlimed sections was 2240 pounds for Tarheel, and the average for these sections was 1711 pounds as against an average of 2991 pounds on the corresponding limed sections. The largest yield of shelled beans on the limed sections was 1279 pounds from Swan, followed in order by Edna with a yield of 1240 pounds, Ohio 9035 with 1136 pounds, Medium Yellow with 1088 pounds, Claud with 1064 pounds, and Manchu with 1044 pounds. All other varieties gave a yield less than 1000 pounds. The highest yield



of shelled beans on the unlimed section was 792 pounds from Medium Yellow, and the second highest 672 pounds from Hollybrook. The average yield from these sections was 553 pounds as against an average of 943 pounds from the corresponding limed sections.

Here is a difference of 390 pounds of shelled beans (6.5 bushels) in favor of the limed sections. In addition to this the same plots yielded 1280 pounds more of dry stalks than the corresponding unlimed plots. These could be used in making manure or could be returned directly to the land to be plowed under.

The following varieties from the limed sections show a nitrogen content in the dry beans of 6.66 per cent or more: Swan, Hollybrook, Ebony, and Baird. The following show a nitrogen content of above 6 per cent, but less than 6.66 per cent: Ito San, Guelph, Manchu, Black Eyebrow, Edna, Medium Yellow and Tarheel. The highest percentage of nitrogen in those from the unlimed sections is found in the Hollybrook, 6.148 per cent. The average nitrogen content of the shelled beans from the unlimed section is 5.917 per cent and the average from the corresponding limed sections is 6.407, a difference of one-half per cent nitrogen, equivalent to 3 per cent protein, in favor of the limed sections.

The percentage of nitrogen in the dry beans this year is somewhat lower than in 1914, the average for that year being 6.205 per cent for the unlimed sections and 6.702 per cent for the corresponding limed sections. The average yield of shelled beans is higher this year than last.

Determinations of the nitrogen in the vines were not made, but it was shown in work reported one year ago that liming does increase the nitrogen content of vines. In the case of non-leguminous crops such nitrogen must come largely from commercial fertilizers and manures or from the store of soil organic matter, but in the case of a leguminous crop much of this nitrogen is taken from the air and thus becomes a definite contribution to the nitrogen store of the soil.

Of the 1915 crop, Black Eyebrow, Ito San, Manchu, Manhattan and Ebony matured in about 120 days from date of planting. Claud, Guelph, Ohio 9035, Wilson, Swan, Medium Yellow and Edna matured in about 135 days, while Tarheel and Hollybrook required 150 days, the former not having fully matured in this time.

In some cases those varieties that gave the largest yield of total dry matter also gave the largest yield of shelled beans. In other cases a low yield of total dry matter was accompanied by a rather high yield of shelled beans, as for example, Manchu. The reverse is true of the Tarheel, which is a slow growing late variety and should not be depended upon to mature seed. If, however, the growing season is long and the ultimate aim is to obtain a large amount of organic matter for soil improvement, this variety promises well. Swan and Claud are likewise rank growers.

# DIASTASE ACTIVITY AND INVERTASE ACTIVITY OF BACTERIA.\*

By GEORGE P. KOCH,

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In the production of nitrogen compounds for consumption by plants, and in the decomposition of carbohydrates by bacteria in the soil, very complicated chemical reactions take place. Enzymes are secreted to effect certain changes; likewise acids, bases, and other compounds are formed. It is with these first mentioned properties that this paper primarily deals. It has been shown by a number of investigators that microorganisms during their life cycle secrete different enzymes. This study is, however, limited to the diastases and invertase.†

That bacteria have the property of secreting certain starch dissolving\* ferments was shown by Wortman (30) in 1882. Observing that bacteria were able to cause a change in starch paste and starch grains as well as in soluble starch, he concluded that the action of bacteria upon starch is through the same process, namely by a ferment such as diastase which is soluble in alcohol and water. In 1890 Fermi (6) recorded the examination of many species of bacteria for diastase and found that the Prioi's bacillus, Koch's *cholera vibrio*, *bacillus ramosus*, *bacillus megaterium* and a spirillum of cheese, gave a positive test for diastase. Cavazzani (3) in 1893 while working with an organism which he identified as probably being *bacillus maydis*, found that this organism had the ability to convert starch into glucose. That fungi as well as bacteria secrete a starch destroying enzyme was shown by Kohnstamm (17) in 1901. He studied the enzyme activity of wood destroying fungi and obtained a starch liquifying amylose from three of these.

That microorganisms secreted a sucrose-inverting ferment was already demonstrated by Borquelot (2). Later Beijerinck (1) in 1885 showed the inverting ability of *sacch. Kephir* and *sacch. Trycola*. He also showed that these organisms cause a change in cane as well as in grape sugar. It is recorded that Fermi (6) examined sixty-two microorganisms for inverting power, only two of which he found possessed this property. In 1890 Kellner, Mori and Nagaoka (14) wrote of the inverting ability of *eurotinus oryzae*. Sclavo (24) who in 1890 studied the biological properties of several bacteria, found that but few usually had the inverting power when grown upon a sugar-free nutrient bouillon. Prob-

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† The thanks of the writer are expressed to Dr. J. G. Lipman for many suggestions which he has supplied throughout the study of this series of problems.

ably the most extensive work on the inversion of cane sugar by microorganisms was accomplished by Fermi and Montesano (9). These investigators attempted to find out what organisms cause the inversion of cane sugar. They also, studied the influence of the reaction of the nutrient solution upon inversion by invertase, and the effect of the presence of the different sugars on inversion. Other factors concerning invertase that these investigators demonstrated were, the time at which invertase is present after the introduction of the sugar, what organisms secrete invertase in the absence of egg albumen, the retention of invertase on a porcelain filter, the influence of heat upon microbes, and the retention of invertase in dialyzing. Of the seventy microbes examined which had been grown on a sugar-free bouillon the following organisms showed invertase activity: *bacillus megaterium*, *bacterium kiliense*, *proteus vulgaris*, *bacillus fluorescens liquif*, a cholera vibrio, *vibrio Metschnikovii*, a white yeast, and a red yeast. It was found also that the quantitative production of invertase was greater with the schizomycetes than with the schistomycetes.

More recent work is recorded by Grezes (10), who in 1912 showed that *Aspergillus niger* produced invertase in much larger quantities in the presence of sucrose and also that it still retained its inverting ability after it had been cultivated for sixty generations in a medium undesirable for invertase formation.

It seems very probable that some of the microorganisms of the soil secrete hydrolytic ferments during their life processes. Yet Fermi (8) in 1910 did not find any of the above mentioned enzymes in the soil samples which he examined. In 1911, however, Schreiner and Sullivan (23) demonstrated that hydrolytic, as well as proteolytic and cytolytic ferments exist in the soil. No doubt some of these ferments are the result of microbial activity and play an active rôle in carbohydrate destruction, and indirectly, in liberating other plant food elements.

Since it has been demonstrated by several investigators that bacteria do secrete diastases and have the property of inverting cane sugar, it was thought that more data bearing upon these points would not be amiss. Consequently these experiments were undertaken:

- (1) To find out whether sufficient diastases and invertase were secreted by bacteria so that they could be quantitatively determined.

- (2) To study the variation in the enzyme (diastase and invertase) secretion by organisms developed in culture solutions of different composition.

- (3) To note the quantitative variation of enzyme secretion by bacteria at different periods.

- (4) To ascertain if there is any direct correlation between enzyme secretion by bacteria and their property of decomposing proteins.

(5) To study the enzyme activities of various organisms and their ability to decompose proteins.

(6) To determine, if possible a correlation between the secretion of enzyme and the following: decomposition of proteins by bacteria, the property of the cultural solution to rotate the plane of polarized light, the percentages of reducing compounds, the formation of acid and the numbers of organisms; and also the inter-correlation which exists among the factors last named.

SECRETION OF ENZYMES, APPARENTLY DIASTASE AND INVERTASE, BY BACTERIA WHEN DEVELOPED IN DIFFERENT CULTURE SOLUTIONS.

The method of determining the diastase activity of extracts in which bacteria had been grown was the one proposed by Thatcher and Koch (28) for determining quantitatively the diastases of plant products. It was, however, not necessary to extract the enzymes since they were already in solution. Briefly, the method is as follows: The acidity of the enzyme solution is first determined by titrating a portion of the hot solutions with N/50 alkali. Then to 25 c.c. of a 10 per cent Lintner (18) soluble starch solution of known acidity, enough N/10 alkali is added so that upon introducing 20 c.c. of the extract the resulting solution, is of the optimum acidity for diastatic activity. This, as according to Effront (4), is 3 mg. of hydrochloric acid in 100 c.c. of solution. After the alkali has been added, 20 c.c. of the enzyme solution is introduced. The temperature of the solution is then quickly brought to 40° C. for a period of 30 minutes. Incubating at 40° C. for 30 minutes furnishes the standard conditions for diastatic activity recommended by Sherman, Kendall and Clark (25). At the expiration of the above mentioned time the enzyme activity was stopped by bringing the acidity of the solution to N/200 acid as recommended by Swanson and Calvin (27). After cooling the solution to 20°-22° C. the proteins were precipitated and removed from the solution. An aliquot of the filtered solution was taken for reduction with a definite volume of Fehling's solution and the amount of reduced copper determined by the iodine method as perfected by Peters (20).

With every determination a blank was carried through the same process in which the enzyme activity was stopped as soon as the 40° C. temperature was reached. The difference between the amount of copper reduced in the determination and that in the blank was considered due to the diastases secreted by the bacteria.

The determination of the quantity of invertase secreted by bacteria was similar to the method used for the diastases, with the exception that the enzymes were allowed to act upon a 10 per cent solution of sucrose at 55° C. Kjeldahl (16) concluded that at 52.5° C. the inversion process proceeds with greater rapidity and Effront (5) states, that the optimum

temperature according to different authors was found to be between 50° and 56° C. It was also found by Thatcher and Koch that 55° C. was the optimum temperature for invertase activity. The enzyme activity was stopped by boiling the solution 5 minutes. This method was found satisfactory by Thatcher and Koch\* and in preliminary work which was performed in this laboratory.

In order to ascertain if diastases and invertase were secreted by bacteria in quantities sufficient to determine quantitatively and also to study the enzyme (apparently diastases and invertase) secreted by bacteria in culture solutions of different composition, culture solutions were inoculated with *bacterium mycoides* and *bacillus subtilis*. The bouillon employed contained 10 gm. of peptone, 5 gm. of sodium chloride, and 5 gm. of Liebig's beef extract to a liter of distilled water. The acidity of the solution was corrected to 1 per cent or N/100 hydrochloric. A series of 99 c c portions of the above bouillon, a series of 99 c c of the same bouillon with an addition of 1 per cent sucrose, and a third containing 1 per cent Lintner's soluble starch were inoculated with 1 c c of a three-day-old culture of *bacterium mycoides*. In like manner portions of the different bouillons were inoculated with *bacillus subtilis*. After incubating for a period of 5 days at 25° C. the solutions were examined for diastase and invertase activity. The solutions were always tested for purity by plating 1 c c. on "synthetic" agar (19). In every instance the heavy growth of bacteria was filtered off before making the enzyme determinations. The determinations were always made in duplicate.

TABLE I

THE EXTRA CELLULAR ENZYME ACTIVITY, APPARENTLY OF DIASTASE AND OF INVERTASE, SECRETED BY BACTERIA OF VARYING ACTIVITY IN CULTURE SOLUTIONS AFTER A FIVE DAY INCUBATION PERIOD

	Diastase gm Cu in 100 c c Solution		Invertase gm Cu in 100 c c Solution	
	Duplicate Determinations	Average	Duplicate Determinations	Average
<i>Bacillus Subtilis</i>	0000		1152	
Bouillon	0075 2531	0037	1227 1753	1189
Sugar Bouillon	2655 0812	2593	1931 1504	1842
Starch Bouillon	0710	0761	1253	1378
<i>Bacterium Mycoides</i>	0176		1604	
Bouillon	0309 0000	0242	1604 2381	1604
Sugar Bouillon	0000 0251	0000	2755 2705	2568
Starch Bouillon ..	0201	0226	2705	2705

\* In unpublished data

From the above table it is apparent that the bacteria have the property of secreting ferments in the various solutions in which they have developed, and the amount secreted is great enough for quantitative determination by the proposed methods. In the following discussions the enzyme activities will be referred to as diastase activity and invertase activity, although it has not been established beyond question that these alone are responsible for the changes effected in the solutions employed. That there is a considerable variation in the enzyme activity of the cultural solutions has been demonstrated. In the case of *bacterium mycoides* there was considerably more enzyme activity which was determined as invertase secreted in the starch bouillon than in the plain bouillon to which no soluble carbohydrates had been added. The sugar bouillon culture solution which was inoculated with *bacterium mycoides* seemed to show no diastase activity, but on the other hand, there was almost as much invert sugar formed in this solution as the result of activity, presumably of invertase, as there was in the case of starch bouillon. Quite the reverse was true with the cultures of *bacillus subtilis* as there was greater diastase activity and invertase activity in the sugar bouillon than in any of the other solutions inoculated with this organism. With regard to the relative amount of diastase and invertase secreted, with one exception there was considerably more invertase activity than diastase activity.

#### PROTEIN DECOMPOSITION BY BACTERIA IN ITS POSSIBLE RELATION TO ENZYME ACTIVITY AT DIFFERENT PERIODS

Having found that the results of hydrolytic processes of bacteria could be determined by the method proposed, the writer considered it desirable to know at what time in the life cycle of the organism these hydrolytic ferments were secreted, and further if any possible relation existed between enzyme secretion and protein decomposition. Consequently a series of 99 c.c. portions of a 1 per cent sugar bouillon, sterilized and brought to 1 per cent acidity were inoculated with 1 c.c. of a three-day-old culture of *bacterium mycoides* and a similar series with *bacillus subtilis*. Fermi and Montesano (9), as well as other investigators found that a sugared bouillon was the most satisfactory medium for studying invertase activity. It was employed in the experiment cited above. On each of the nine consecutive days determinations for diastases and invertase activity and protein decomposition were made. The following method was employed for measuring protein decomposition. The 99 c.c. contents of the bacterial growth in the bouillon were placed in a copper flask, 100 c.c. distilled water added, and the ammonia distilled off by the addition of about 10 gm. of magnesium oxide. The distilling was regulated to deliver 100 c.c. in 40 minutes. This method was found to be satisfactory.

TABLE II  
THE VARIATION IN THE HYDROLYTIC PROCESSES AND THE PROTEIN DECOMPOSITION BY BACTERIA AT DIFFERENT PERIODS.  
A—BACTERIUM MYCOIDES

Days after Inoculation	Diastase gm Cu in 100 c.c. Solution		Invertase gm Cu in 100 c.c. Solution		Ammonia produced in a 100 c.c. Solution, mg N	
	Duplicate Determinat'ns	Average	Duplicate Determinat'ns	Average	Duplicate Determinat'ns	Average
1	0101	0113	2909	2898	1 17	1 21
	0126		2887		1 25	
	0405		0354		1 20	
2	0430	0417	0455	0404	1 30	1 25
	0304		— 0050		2 20	
3	0254	0279	0000	— 0025	2 22	2 21
	— 0278		1800		lost	
4	— 0227	— 0252	3815	3807	2 94	2 94
	0050		— 1443		lost	
5	0000	0025	lost	— 1443	4 42	4 42
	0000		0254		8 97	
6	— 0025	— 0012	0229	0241	7 97	8 47
	— 0331		0458		8 13	
7	lost	— 0331	0636	0547	7 25	7 69
	0420		— 0356		10 50	
8	0229	0324	— 0611	— 0483	12 14	11 32
	0458		— 0433		12 40	
9	0458	0458	— 0203	— 0318	10 36	11 38

B—BACILLUS SUBTILIS

Days after Inoculation	Diastase gm Cu in 100 c.c. Solution		Invertase gm Cu in 100 c.c. Solution		Ammonia produced in a 100 c.c. Solution, mg N	
	Duplicate Determinat'ns	Average	Duplicate Determinat'ns	Average	Duplicate Determinat'ns	Average
1	0607	0607	3087	3051	64	63
	0607		3016		62	
	0253		0126		81	
2	0455	0354	0007	0066	85	83
	— 0152		— 0152		1 44	
3	— 0101	— 0126	0000	— 0076	1 52	1 48
	— 0177		3853		2 26	
4	— 0227	— 0204	3752	3802	3 00	2 63
	0050		— 1265		2 60	
5	— 0025	0025	— 1342	— 1303	2 66	2 63
	0433		0101		4 61	
6	0407	0420	— 0076	0012	3 65	4 13
	0636		0611		4 67	
7	0458	0547	0791	0701	5 09	4 88
	0878		— 0254		6 88	
8	0611	0744	— 0203	— 0228	6 29	6 58
	0839		— 0178		7 83	
9	0687	0763	— 0484	— 0331	8 41	8 12

Upon examining the above table it becomes apparent that the processes measured as diastase activity and invertase activity by bacteria in sugar bouillon vary greatly from day to day. Likewise there is a considerable difference in the secretion of these ferments by different organisms. Figures 1 and 2 express this to a much better advantage. In the case of *Bacterium mycoides* a maximum diastase activity is reached on the second

day, after which there is a decrease. This fact is also recorded by Cavazani (3), who states that on the following day the organism had lost much of its diastase activity. A negative determination of diastase appears on the fourth day, a fact which according to the data at hand, indicates that bacteria are not only capable of secreting hydrolytic ferments for the purpose of acting upon carbohydrates but likewise are able to produce a condition which prevents their hydrolysis. In order to simplify the terminology in this discussion, this negative activity, the exact nature of which has not been determined, will be arbitrarily referred to as resulting from a "contra" enzyme. The solutions showed slight diastase activity on the fifth day, a "contra" enzyme action again on the sixth and seventh, a marked activity on the eighth, and the maximum on the ninth day. In the case of the diastatic properties of *bacillus subtilis*, a marked activity was shown the first day, with a gradual decrease thereafter, so that on the third day a negative result was obtained. The fourth day shows a greater "contra" enzyme activity and then follows a gradual increase in extra-cellular diastase. On the ninth day, as in the case of *bacterium mycoides* the maximum activity was attained. Upon examining the text figure one sees that the diastase activity of the organisms varies considerably.

With regard to the extra-cellular invertase of these two bacteria, it is noted that in this respect the activities of these organisms were similar; as in both cases there was considerable invertase activity the first day with a decrease until a negligible degree was shown on the third day, the maximum on the fourth, and a "contra" enzyme action on the fifth day equal to one-third the activity on the previous day. Again there was considerable invertase activity on the seventh with a "contra" invertase activity on the eighth and ninth days.

With both organisms the maximum invertase activity was about six or seven times that of the diastases. Likewise the maximum "contra" invertase activity was four to six times that of the "contra" diastase activity. However, it is possible that these experiments were not repeated a sufficient number of times to demonstrate that these phenomena of "contra" enzyme activity always occur under conditions of the experiment.

Upon noting the text figures showing the decomposition of the proteins, i. e. formation of ammonia, it is apparent that with both organisms there was a gradual increase in the decomposition of the proteins up until the ninth day. From these data there seems to be no correlation between the secretion of the hydrolytic ferments and the production of ammonia due to the protein decomposition.

#### THE POSSIBLE RELATION OF ENZYME ACTIVITIES OF BACTERIA AND THEIR ABILITY TO DECOMPOSE PROTEINS.

Since there was considerable irregularity in the secretion of hydrolytic ferments by *bacterium mycoides* and *bacillus subtilis* and since no



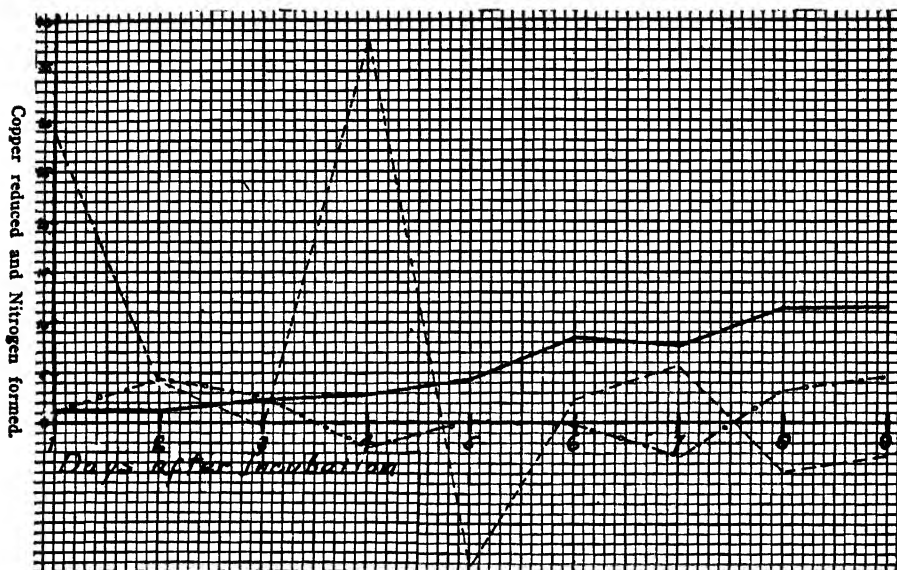


FIGURE 1.—THE VARIATION IN THE HYDROLYTIC PROCESSES AND THE PROTEIN DECOMPOSITION BY BACTERIUM MYCOIDES AT DIFFERENT PERIODS.

----- Invertase—Each space represents .01 gm. Copper reduced.  
 —o—o— Diastase—Each space represents .01 gm. Copper reduced.  
 ————— Ammonia—Each space represents 1 mg. Nitrogen.

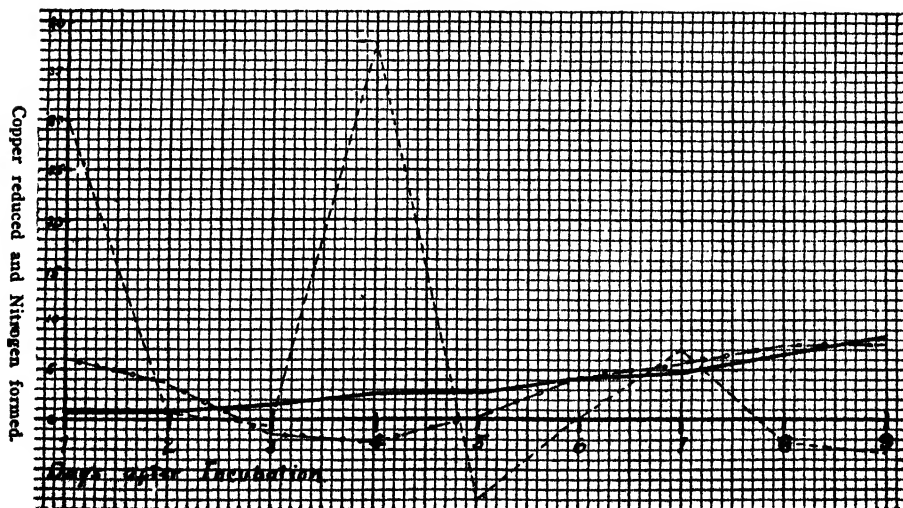


FIGURE 2.—THE VARIATION IN THE HYDROLYTIC PROCESSES AND THE PROTEIN DECOMPOSITION BY BACILLUS SUBTILIS AT DIFFERENT PERIODS.

----- Invertase—Each space represents .01 gm. Copper reduced.\*  
 —o—o— Diastase—Each space represents .01 gm. Copper reduced.  
 ————— Ammonia—Each space represents 1 mg. Nitrogen.

\* Errata:—The curve should read —.1303 on the fifth day, instead of —.0803.

correlation could be seen between the secretion of these ferments and protein decomposition, it was desirable to find out if any correlation existed between enzyme secretion and protein decomposition produced by other common soil organisms. In a manner similar to the methods previously used, sterile sugar bouillon was inoculated with three-day-old cultures of the different organisms and after being incubated for one day at 25° C. the inoculated culture solutions were examined for diastase and invertase activity and protein decomposition.

TABLE III.  
THE ENZYME ACTIVITIES OF VARIOUS ORGANISMS, AND THE PROPERTY TO DECOMPOSE PROTEINS IN ONE DAY.

Kind of Organism	Diastase in 100 c.c. Solution, gm. Cu.		Invertase in 100 c.c. Solution, gm. Cu.		Ammonia produced in 100 c.c. mg. N.	
	Duplicate Determin's	Average	Duplicate Determin's	Average	Duplicate Determin's	Average
Bacterium Mycoides. . . 1.	.0000		.0335		5.80	
" " . . . 2.	.0038	.0019	.0360	.0347	5.94	5.87
" " . . . 3.	.0388		.0258		.83	
" " . . . 3.	.0000	.0194	.0181	.0219	.85	.84
" " . . . 3.	.0000		.0646		3.11	
" " . . . 3.	.0000	.0000	.0129	.0389	3.11	3.11
Bacillus Subtilis . . . 1.	.0155		— .0621		3.78	
" " . . . . . 2.	.0233	.0194	— .0697	— .0659	3.82	3.80
" " . . . . . 2.	.0190		— .0181		3.97	
" " . . . . . 3.	.0155	.0172	— .0362	— .0271	3.93	3.95
" " . . . . . 3.	— .0233		— .0258		.47	
" " . . . . . 4.	— .0310	— .0272	— .0206	— .0232	.45	.46
" " . . . . . 4.	.0794		— .2192		1.98	
" " . . . . . 4.	.0970	.0882	— .1965	— .2078	2.00	1.99
Bacillus Coli . . . . . 1.	— .0155		— .1419		3.26	
" " . . . . . 2.	— .0384	— .0219	— .1625	— .1522	3.26	3.26
" " . . . . . 2.	.0192		— .0335		3.59	
" " . . . . . 2.	.0116	.0154	— .0206	— .0270	3.69	3.64
Bacillus Cereus . . . . .	.0078		.0181		2.66	
Bacillus Megaterium . . . . .	— .0038	.0019	.0051	.0116	2.70	2.68
Bacillus Megaterium . . . . .	— .0190		— .0776		.35	
Bacillus Megaterium . . . . .	— .0697	— .0443	— .0335	— .0556	.37	.36
Bacterium Vulgaris . . . . .	.0116		— .0466		4.57	
Bacterium Vulgaris . . . . .	.0310	.0213	— .0438	— .0452	4.57	4.57
Bacillus Cholera Suis . . . . .	— .0384		lost		3.01	
Bacillus Cholera Suis . . . . .	— .1163	— .0773	.0906	.0906	3.05	3.03
Bacillus Fluorescens Liqui . . . . .	.0038		.1865		.87	
" " " " . . . . .	— .0116	— .0038	.1835	.1850	.91	.89
" " " " . . . . .	.0038		— .2096		2.62	
" " " " . . . . .	— .0078	— .0019	— .2310	— .2203	2.62	2.62
Bacillus Vulgatus . . . . .	.0384		lost		.37	
Bacillus Vulgatus . . . . .	.0697	.0540	.0517	.0517	.39	.38
Bacillus Proteus Vulgaris . . . . .	— .0116		— .1990		4.86	
Bacillus Proteus Vulgaris . . . . .	.0038	— .0038	— .2580	— .2285	4.96	4.91

The data in Table III show again that there is no direct correlation between secretion of hydrolytic "enzymes" and ammonia production. For instance, in the case of *bacterium mycoides* 2, which produced .84 mg. of ammonia, the diastatic activity is indicated by .0194 gm. of copper and the invertase activity by .0219 gm. of copper, while *bacillus fluorescens*

*liquifaciens* which produced almost the same quantity of ammonia, showed a diastase activity of  $-.0038$ , and invertase activity of  $+.1850$  gm. of copper. Similar cases are noted upon the comparison of *bacillus cereus* with *bacillus fluorescens liqui* and *bacillus megaterium* with *bacillus vulgatus*. These organisms had a property of decomposing proteins similarly but their hydrolytic enzyme activities were entirely different. In like manner different pure cultures of the same species of organisms varied greatly in ammonia production as well as in enzyme secretion.

#### PROTEIN AND CARBOHYDRATE DECOMPOSITION BY BACTERIA IN ITS POSSIBLE RELATION TO ENZYME ACTIVITY.

Previous experimentation demonstrated the irregularity in the enzyme activity of the different bacteria and an absence of any possible correlation existing between the production of hydrolytic enzymes and protein decomposition. The next question which arises is whether there is any correlation between enzyme activity and the sugar content, presence of reducing compounds, formation of acid, numbers of organisms, and the amount of protein present. Two organisms, *bacillus coli* and *bacterium mycoides*, of entirely different morphological character and habitat, were studied for these properties. As in previous work sterile sugar bouillon solutions were inoculated with three-day-old cultures of the respective organisms. Daily determinations of the diastase and the invertase activities, optical properties, presence of "reducing" compounds, acid formation, numbers of organisms, and ammonia accumulations were made for a period of eight days.

The property of the solution of rotating the plane of polarized light was determined by first removing the proteins from the solution and then taking the reading directly by means of a saccharimeter.

The reducing compounds were determined by boiling an aliquot of the protein-free bacterial extract with a standard Fehling's solution and then applying the iodine titration method which was used in the enzyme procedure. The acid present was found by titrating an aliquot of the hot solution with N/50 alkali.

The bacterial counts were made upon synthetic agar on the fifth day after the plates were poured.

Upon examining Table IV and Figures 3 and 4, one sees at a glance that there is a considerable irregularity of hydrolytic enzyme secretion: on the second day there was considerable diastase activity which was almost similar to that tabulated in Table II. After a marked decrease in activity for two or three days the maximum was reached on the fifth day with *bacterium mycoides* and on the sixth with *bacillus coli*. With the exception of *bacillus coli*, which on the eighth day produced  $-.0301$  gm. of copper, there were no other examples of "contra" diastase activity by these two organisms. As it was previously demonstrated,

TABLE IV.

DIASTASE AND INVERTASE SECRETION BY BACTERIA, THE DECOMPOSITION OF PROTEINS, THE ROTATION OF THE SOLUTION, THE FORMATION OF ACID AND THE NUMBERS OF ORGANISMS, PRESENT DAILY IN SUGAR BOUILLON INOCULATED WITH DIFFERENT ORGANISMS FOR A PERIOD OF EIGHT DAYS.\*

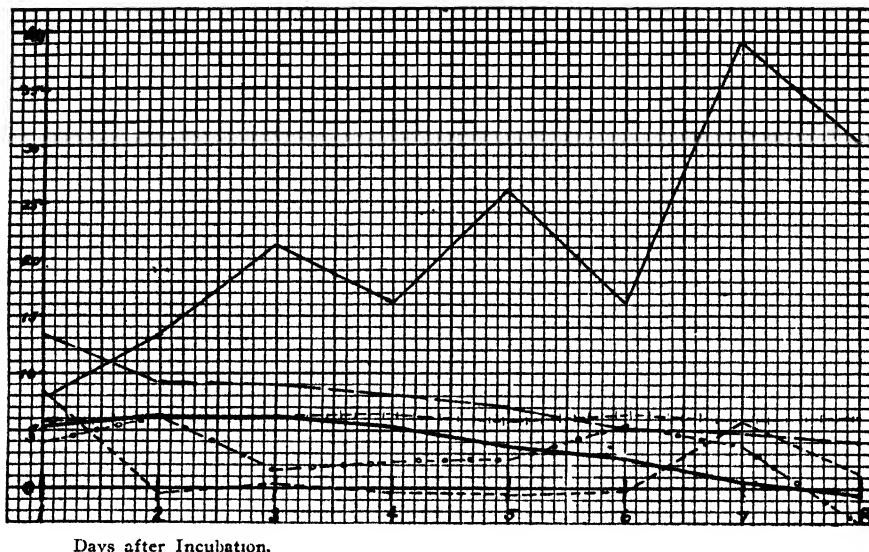
A.—BACILLUS COLI.

Days after Inoculation	Diastase in 100 c.c. Solution gm. Cu.		Invertase in 100 c.c. Solution gm. Cu.		Ammonia prod'd in 100 c.c. Sol., mg. N.		Saccharimeter readings % of Sugar	Acid present in 100 c.c. gm. HCl	Numbers of organisms in 1 c.c.
	Duplicate Determin's	Average	Duplicate Determin's	Average	Duplicate Determin's	Average			
1	.0292		.0390		5.29				
	.0488	.0390	.1341	.0865	5.28	5.26	1.33	.0583	728,500,000
	.0624		— .0065		6.28				
2	.0585	.0604	.0000	— .0032	6.40	6.34	.92	.0590	1,333,000,000
	.0097		.0065		6.14				
3	.0234	.0165	.0000	.0033	6.14	6.14	.90	.0619	2,125,000,000
	.0292		— .0234		5.45				
4	.0156	.0224	— .0130	— .0182	5.53	5.49	.81	.0641	1,625,000,000
	.0215		— .0026		3.69				
5	.0254	.0234	— .0039	— .0033	3.79	3.74	71	.0597	2,592,000,000
	.0488		— .0208		2.40				
6	.0644	.0566	— .0247	— .0227	2.58	2.49	.52	.0634	1,512,000,000
	.0449		.0637		.61				
7	.0292	.0370	.0521	.0579	.63	.62	.49	.0584	3,888,000,000
	— .0527		.0168		— .44				
8	— .0075	— .0301	.0078	.0123	— .49	— .41	.39	.0616	3,024,000,000

B.—BACTERIUM MYCOIDES.

Days after Inoculation	Diastase in 100 c.c. Solution gm. Cu.		Invertase in 100 c.c. Solution gm. Cu.		Ammonia prod'd in 100 c.c. Sol., mg. N.		Saccharimeter readings % of Sugar	Acid present in 100 c.c. gm. HCl	Numbers of organisms in 1 c.c.
	Duplicate Determin's	Average	Duplicate Determin's	Average	Duplicate Determin's	Average			
1	.0175		.1054		7.36				
	.0156	.0165	.0872	.0963	7.36	7.36	1.33	.0561	82,150,000
	.0565		.0182		13.71				
2	.0546	.0556	.0130	.0156	13.89	13.80	1.32	.0459	86,800,000
	.0390		— .0065		17.90				
3	.0156	.0273	— .0156	— .0110	17.90	17.90	1.57	.0433	186,000,000
	.0332		.0091		21.20				
4	.0156	.0244	.0117	.0104	21.30	21.25	2.03	.0459	137,500,000
	.0742		.0390		25.98				
5	.0624	.0683	.0403	.0396	24.22	25.10	2.59	.0372	89,640,000
	.0429		.0208		26.43				
6	.0315	.0372	.0078	.0143	28.65	27.54	3.17	.0284	71,925,000
	.0449		.0195		28.98				
7	.0273	.0376	.0065	.0130	30.82	29.90	3.26	.0251	54,000,000
	— .0156		.0585		31.36				
8	.0195	.0019	.0390	.0487	32.00	31.68	3.30	.0277	54,000,000

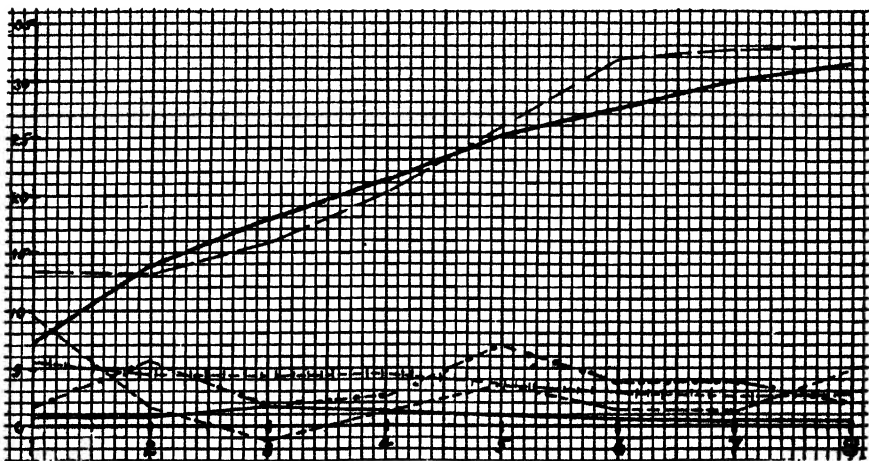
\* In the case of the saccharimeter reading, an average of five determinations was recorded. Acidity readings represent an average of four determinations; while the bacterial counts were made in triplicate.



Days after Incubation.

FIGURE 3.—STUDIES ON THE ENZYME ACTIVITY OF *BACILLUS COLI* FOR A PERIOD OF EIGHT DAYS.

- Invertase—Each space represents .01 gm. Copper reduced.
- o—o— Diastase—Each space represents .01 gm. Copper reduced.
- Ammonia—Each space represents 1 mg. Nitrogen.
- Sacch. Readings—Each space represents .1% Sugar.
- |—|— Acid—Each space represents .01 gm. HCl.
- ..... Numbers of Organisms—Each space represents 100,000,000.



Days after Incubation.

FIGURE 4.—STUDIES ON THE ENZYME ACTIVITY OF *BACTERIUM MYCOIDES* FOR A PERIOD OF EIGHT DAYS.

- Invertase—Each space represents .01 gm. Copper reduced.
- o—o— Diastase—Each space represents .01 gm. Copper reduced.
- Ammonia—Each space represents 1 mg. Nitrogen.
- Sacch. Readings—Each space represents .1% Sugar.
- |—|— Acid—Each space represents .01 gm. HCl.
- ..... Numbers of Organisms—Each space represents 100,000,000.

there was a considerable amount of invertase secreted on the first day after inoculation. This was followed by a great decrease in activity, with a "contra" invertase activity followed by a steady increase and another decrease. On comparing the enzyme activity with the other products of the bacterial functions it is apparent that there is no correlation between them. With respect to the saccharimeter readings, the activities of the organisms were entirely different. In the solutions of *bacillus coli*, the saccharimeter readings were steadily on the decrease, while in the case of *bacterium mycoides* the reverse was true. The saccharimeter reading of solutions inoculated with *bacterium mycoides* on the first day was 1.33 per cent while on the eighth it was 3.30 per cent. The above shows that while the *bacillus coli* had utilized the greater percentage of sugar on the eighth day, *bacterium mycoides* had increased the sugar content or at least increased the saccharimeter reading at that time. A similar circumstance was observed in the protein decomposition, noted in the table above and in the text figure. In the case of *bacillus coli*, decomposition took place up to the second day while thereafter the protein formation was greater than the decomposition, so that on the eighth day there was slightly less free ammonia in the bacterial culture solution than there was in the original blank solution which had not been inoculated. This is no doubt due to the fact shown by Kendall (15) that the *bacillus coli* with the presence of a considerable amount of carbohydrates which are readily utilizable may protect the nitrogen compounds from attack by the organisms. In the case of *bacterium mycoides* there was a gradually increased protein decomposition up to and including the eighth day. It is very apparent that there is no direct correlation between the secretion of hydrolytic ferments, the saccharimeter readings and the protein decomposition. There seemed to be a direct correlation between utilization of carbohydrates, protein decomposition and the amount of acid formed. In the case of *bacillus coli* there was an almost steady increase of acid, showing that the carbohydrate decomposition was more rapid than the protein decomposition. In other words, more acid was liberated from carbohydrate decomposition than could be neutralized by the ammonia set free as the result of the protein decomposition. With *bacterium mycoides* the process was entirely reversed as in this case there was a continuous increase in the saccharimeter readings, the acid content steadily decreased while there was a continuous increase in ammonia. With both bacteria there was a gradual increase in numbers until the third day, after which there was a decrease in the *bacterium mycoides* cultures. This was probably due to the "dying out" of some of the organisms. The data in the text figure show a large secretion of invertase on the first day. In comparing this fact with the numbers of organisms present, it is probable that the rapid multiplication of the organisms caused the large secretion of invertase. In the case of *bacillus coli* this increase was from 1,199,700,000 to 72,850,000,000.

The determinations of the presence of reducing compounds demonstrated that the bacteria do not produce an excess of extra-cellular reducing substances. In all cases the blanks showed as much invert sugar as did the determinations. The fact that no invert sugar is present in a culture solution of sugared bouillon after the first or second days was pointed out by Fermi and Montesano (9). Hence the question at once arises, what part do these extra-cellular diastases play in the solution if they do not hydrolize organized compounds and make them more accessible for the organisms?

With regard to the acidity of the solution and the activity of diastases secreted by bacteria, it has already been found by Wortman (30), that the ferment secreted by the bacteria is able to act upon a solution containing starch when the solution is neutral and that in a weak acid solution the enzyme activity is increased. Likewise Fermi and Montesano (9) recorded that when the reaction of the sugar-free bouillon changes, some of the microbes lose this property and in slightly sugared bouillon all of them with the exception of *Vibrio Metschnikvii* retain their inverting property. It was found by Thompson and O'Sullivan (29) that invertase is very sensitive to acids. Hudson (11) observed that in alkaline solutions invertase shows no activity, while in the weakly acid solutions its enzymatic power reaches a maximum after which it decreases with increasing acidity. Since the acidity of these media was ten times greater than the degree of acidity which Effront (4) found to be optimum for diastatic activity, it seems that in solutions of as high concentration of hydrogen-ions as these (1 c.c. of the solution contained .0003645 gm. of hydrochloric acid) diastase formation would be impossible. Hudson (12) in 1908 found that the acidity for optimum invertase activity was 6N/10000 hydrochloric acid. Sorensen (26) in 1909 noted that the greatest activity was reached when the solution had the hydrogen-ion concentration of  $10^{-4.4}$  to  $10^{-4.6}$ . Preliminary work by the writer demonstrated that there was little difference between the invertase activity when the acidity of the solution was 6N/10000 and that when the solution was 3N/10000 hydrochloric acid. Consequently there was almost twenty times as much acid in the solutions in which the bacteria developed as has been found to be the optimum acidity for invertase activity. Hence the question presents itself, do the extra-cellular enzymes secreted by the bacteria, whose optimum development is in solutions of N/100 acid (hydrochloric), function in the bacterial processes at such a high concentration of hydrogen-ions?

That protein compounds exhibit considerable influence upon the property of bacteria to secrete hydrolytic ferments has been shown by Cavazzani (3) in his experiments with *bacillus maydis*. In the absence of protein he got only a trace of diastase activity, while in the presence of egg albumen, diastase activity equivalent to 0.115 gm. of glucose took place. In like manner Fermi and Montesano (9) showed that microbes

form invertase in the presence of egg albumen. Probably the most direct data available regarding the effect of the presence of protein upon enzyme activity is the work recorded by Rosenthaler (21). He shows that  $\delta$  and  $\sigma$  emulsion exerts a protective action for the hydrolytic enzymes against alkali. Saito (22) also states, in work with *Aspergillus oryzae*, that nitrogenous bodies are contributing factors to the formation of diastase. Hence, no doubt, the presence of an abundance of protein compounds in these experiments exerted a protective influence against the attack of the acid. But in the case of *bacillus coli*, as shown in Table IV and in Figure 3, this organism increased the protein content of the medium after the third day, while the diastase activity and the invertase activity of the medium were not substantially different from those of *bacterium mycoides* which continually decomposed the proteins. Likewise the media in which *bacillus coli* developed continually increased in acid; but the harmful factors resulting from this increase in activity were probably neutralized by the increase in protein content. In the case of *bacterium mycoides* there was a gradual decrease in acid present and it is probable that the ammonia formed, as well as the presence of protein compounds, played the part of a protective agent against the acid.

The temperature at which the cultures were incubated, 25° C., was much lower than the optimum for activity of diastases and invertase. Several investigators have shown that the hydrolytic enzyme activity increases until the optimum is reached, beyond which point the activity is quickly destroyed. Thus at this temperature of incubation enzyme activity would take place, but not at its maximum rate. It therefore seems probable that the secreted hydrolytic ferments function in the bacterial processes even though, analytically, the conditions are not optimum for the activity of these enzymes.

#### SUMMARY.

The results obtained in the above experiments indicate that:

1. Enzymes determined as diastases and invertase are secreted by bacteria in culture solutions in amounts sufficient to be determined quantitatively.
2. There is considerable variation in the above mentioned activity of enzymes secreted by bacteria when they are developed in cultural solutions of different composition.
3. Enzyme activity (diastase and invertase) of bacteria as determined in this experiment is variable from day to day under conditions otherwise the same.
4. Bacteria appear to have the property of causing a factor which will prevent starch hydrolysis and sucrose inversion.
5. There seems to be no direct correlation between hydrolytic enzyme secretion and protein decomposition by bacteria.



6. Enzyme activity (diastase and invertase) of different species of bacteria varies greatly. Likewise there is a variation in enzyme activity of different cultures of the same species.

7. There is no direct correlation between hydrolytic enzyme secretion and the property of the solution to rotate the plane of polarized light, the percentage of reducing compounds present, the formation of acid and the number of organisms.

8. Bacteria have the property of increasing the rotatory power of a solution as they have of decreasing this property.

9. There is some evidence for a possible correlation between the utilization of the protein decomposition determined as ammonia and the formation of acid.

10. Bacteria do not produce in the solution a surplus of reducing compounds.

11. There is an increase in bacterial numbers up to the third day, after which with *bacillus coli* the numbers are irregular, while with *bacterium mycoides* there is a decrease.

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# SOIL SCIENCE

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## THE LOESS SOILS OF THE NEBRASKA PORTION OF THE TRANSITION REGION :

### I. HYGROSCOPICITY, NITROGEN AND ORGANIC CARBON.<sup>1</sup>

By

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#### INTRODUCTION.

"The sloping plains country lying between the Rocky Mountains and the Mississippi, quite arid at the foot of the mountains, but with rainfall increasing more or less regularly to eastward, forms a transition-belt between the arid and the humid region of which but a small portion<sup>3</sup> has been systematically studied with respect to its soil formation" (18—*Hilgard, Soils*, p. 397).

In this *transition region* no other surface formation seems to offer such an opportunity for the study of the relation of the properties of its soils to the climate as does the large area indicated in Fig. 1 as derived from wind-laid material and commonly referred to as *loess*. The soils of about half of Nebraska are derived from this deposit and the agricultural importance of these far exceeds that of all the other soil areas combined. The Dune Sands which occupy most of the north-central portion of the state are devoted almost exclusively to pasturage. Residual soils, while extensively developed, are almost entirely confined to the distinctly semi-arid western portion of the state. Glacial soils occupy a considerable portion of the southeastern part of the state, but much of their area is too

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<sup>1</sup> Received for publication February 10, 1916.

<sup>2</sup> The work reported in this paper was carried out at the Nebraska Agricultural Experiment Station, where the authors were Chemist and Research Assistant in Chemistry, respectively.

<sup>3</sup> This "small portion" refers to parts of Minnesota and North Dakota.

rough for satisfactory tillage and hence their agricultural importance does not correspond to their acreage. The loess, on the contrary, although in a few places too badly dissected to permit of cultivation, in general forms level plains or comparatively gentle slopes, ideal for tillage, and only a small part of it lies so far to the west as to be very seriously affected by a lack of sufficient rainfall. All of these factors combine to give the loess soils the most prominent place in the study of Nebraska agriculture.

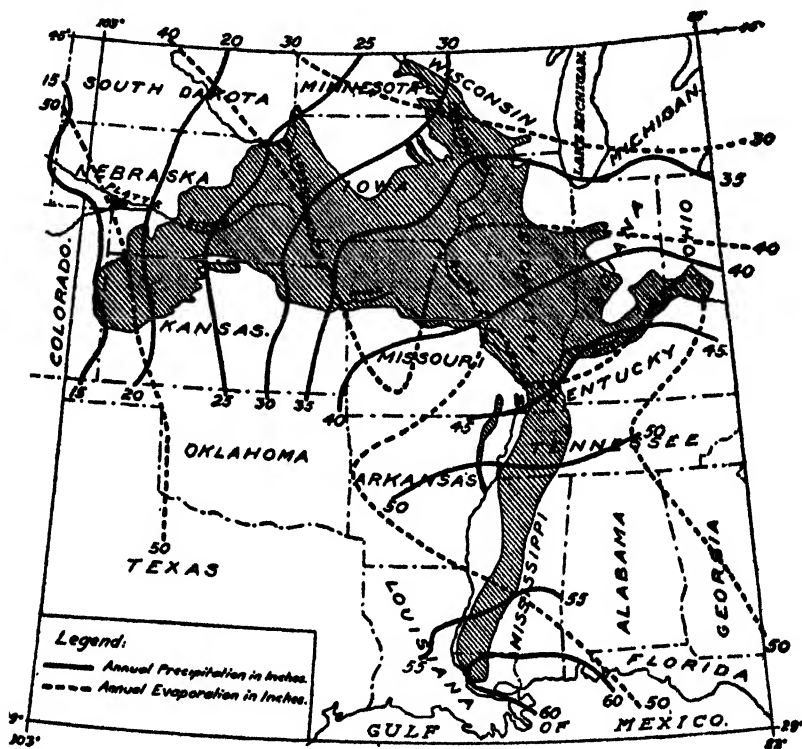


Fig. 1.—Map showing distribution of loess<sup>1</sup> in the United States and also the annual precipitation<sup>2</sup> and evaporation<sup>3</sup> of the loess region. (<sup>1</sup>From map by G. N. Coffey, to accompany Bul. 85, Bur. Soils, U. S. Dept. Agr., 1912, and map of Reconnaissance Survey of Western Nebraska, Bur. Soils, 1913. <sup>2</sup>Climatology of U. S., Plate xxvi, 1906. <sup>3</sup>Monthly Weather Review, 1904, fig. 1, p 558.)

Uniformity in physical properties has long been recognized as characteristic of the loess, but previous to the studies partly reported in the present article little attention had been paid to the chemical composition of the Nebraska portion.

Within only recent years has it come to be recognized, first through the work of Hilgard in the United States, and later through the work of Dokutschajew and Sibertzew in Russia, that in general the character of the soil is much more dependent upon the climate of the region in which it is found than upon the character of the rock from which it has been derived, or upon the manner of its formation. Thus a granite may weather to produce a soil very similar to that developed upon a wind-laid silt loam or a lacustral clay when all three have been exposed for a sufficient length of time to the same climate, while all will be quite distinct in character from the soils that would have resulted under a radically different climate.

According to climate soils are classified as those of *humid regions*, in which the precipitation exceeds the evaporation, and those of *arid regions*, in which the precipitation is less than the evaporation from a water surface (23, p. 523). It is difficult to define sharply the limits for either class. In addition to the amount of the annual precipitation it is necessary to take into consideration its distribution as well as the temperature, the relative humidity of the air, the wind movement and the intensity of the solar radiation. Accordingly, the usual meteorological data do not give us definite information as to the class to which the soils of a region belong. A much better criterion is the amount of percolation which the soil suffers; if the seepage is considerable it is under humid conditions.

The Committee, of the American Society of Agronomy, on Soil Classification and Mapping has recently proposed to recognize a third division to embrace the soils of the *semi-arid* regions (13, p. 285).

The uniformity in physical properties, recognized as characterizing the material of the loess, should tend to produce, under uniform climatic conditions, soils uniform in chemical properties. The importance of working with soils of similar texture in a study of the relation of their chemical composition to climate is evident, as the most marked effect of a heavy precipitation is the leaching out of the soluble salts and the carbonates. A precipitation, too light to cause the water to penetrate beyond the reach of the plant roots in the case of a fine-textured soil, may regularly cause percolation in an adjacent sand, the water-holding capacity of the latter being much lower. There would thus be developed in the former the characteristics of an arid, and in the latter those of a humid soil, although the two types may be adjacent.

The residual soils immediately to the west and northwest, where similar in water capacity, may be expected to resemble those of the adjacent loess. Likewise, the glacial soils of the southeastern part of the state are likely to show many of the characteristics of the loess around Lincoln and Weeping Water.

The mode of deposition of this *aeolian* deposit makes it highly prob-

able that the portion of it now constituting the soil and subsoil was originally very uniform in chemical composition, at least within the limits of different districts, even though it may have shown great variations between distant parts, as when that in Ohio is compared with that in eastern Colorado.

Our study of the loess has been confined entirely to the Nebraska portion, which offers exceptional advantages, the humidity decreasing steadily from the Missouri to its western limit. The formation covers the hills and valleys alike to a depth of from 20 to 100 feet, being much thicker than this in some places and much thinner in others. Throughout the first hundred miles westward from the Missouri it is underlain by Kansan till, while throughout the remainder of the distance it overlies Cretaceous and Tertiary formation (7, p. 169; 14).

The dark-colored prairie soils which occupy the Nebraska portion of the loess have been recognized as similar to the Russian Chernozem (black earth). The chief labors of Russian soil investigators have been devoted to the Chernozem and they emphasize the paucity of data on similar soils in the United States. Thus Kossowitsch (20, p. 338-339) makes the following statements:

"Concerning the Chernozem soils of North America as such we know very little; the American soils investigators, in so far as we know, actually even do not recognize any special soil type which would be analogous to the Russian Chernozem soils. The zone . . . . . of the Chernozem extends approximately through the states of North and South Dakota, Nebraska, Kansas, Oklahoma and Texas, but at present we do not have any at all definite data to make it clear how wide this zone is, and to what extent the representatives of the Chernozem soils occur in it."

\* \* \* \* \*

"Unfortunately we do not have available chemical analyses of the different levels from typical soils of the prairies mentioned. The data of such analyses would make it possible for us to elucidate more fully the peculiarities of these soils and their real nature."<sup>1</sup>

Even for the Chernozem soils of Europe he finds, on assembling the available data, that only very few such analyses have been published, and even these are far from complete. Nabokich (22, p. 203) points out that there is still lacking a knowledge of the exact character of the vertical profile of most of the soil types of Europe, the chemical study of the successive soil levels begun more than thirty years ago by Dokutschajew, Schmidt, Berendt, Müller and others having been neglected by the soils investigators who followed them.

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<sup>1</sup> Author's translation from Kossowitsch, loc. cit.

As no other soils from the regions of summer rains have contributed so much to the study of the relation of soil character to climate as have the Chernozem soils of Russia, both the analytical data and the agricultural history of these are of especial interest in connection with the soils of the transition region. Because of their three most marked characteristics—great fertility, richness in organic matter and wide distribution—they early attracted the especial attention of Russian investigators, and the explanation of their origin has been a matter of controversy for over a hundred years. They occupy the greater part of the southeastern half of European Russia. Toward the north and northwest they pass gradually into the gray, forest-covered soils, there being no sharp line of separation, while on the southeast they assume a chocolate—or chestnut-color and merge into the light-colored soils of the desert areas. Thus with a steady increase in the humidity of the climate the light-colored soils of the southeast pass into those darker in color and these in turn into the typical black soils (Chernozem). With still increasing humidity the latter show a gradual change into the light gray forest soils. The productivity attains a maximum along with the color, the light desert soils being unproductive from lack of rainfall and the light-colored forest soils because of the lack of the essential elements of nutrition.

The climate of the Chernozem zone in Russia resembles that of western Nebraska in that it is cold in winter with a small snow-fall, has hot summers with a dry atmosphere, is subject to sudden changes of temperature, and is characterized by insufficient precipitation. This want of moisture is due less to the amount, 16 to 20 inches, than to its distribution. It falls chiefly during the warm, growing season, and is quickly transpired by the plants or evaporated. Much falls in heavy showers, causing a great loss in the form of run-off. The fineness of texture of the soils increases both the runoff and the evaporation. The natural vegetation is similar to that of our prairies, but Russian investigators report that it is very difficult to now find any really virgin fields.

The Chernozem soils occur chiefly on the loess and there is still found outside of Russia the erroneous view that they are *confined* to this geological formation. Extensive areas occur on the glacial plains, lacustral clays, limestone and crystalline rocks, sufficient evidence that this soil's formation depends upon the climate rather than upon the character of the parent rock. One property possessed in common by all geological formations on which this black soil is typically developed is their ability to produce a fine-textured product on weathering. The topography on which the Chernozem occurs is similar to that of our prairies—almost level to gently rolling.

It is now generally accepted that the grassland vegetation has caused the dark color of the Chernozem soils. The large quantities of roots



left by the plants were not provided with conditions favorable to rapid decay, the soils being throughout most of the year either too dry or too cold. The plants, consisting largely of biennials or perennials rooted deeply, and the roots were of short life compared with those of forest vegetation. Thus large quantities of the dead roots were annually added to the soil. Considerable amounts of the aerial parts of the plants were dragged down by insects or fell into crevices during dry weather. It is not improbable that soluble organic compounds from the aerial parts were carried down into the soil by the rains. In passing from the most arid to the most humid portions of the plains the conditions favored an increased rate of growth but also an increased rate of decay. Up to a certain point the former increased the more rapidly and at that point there is found the maximum accumulation of organic matter.

Kossowitsch (20, p. 333) states that the Chernozem soils, both in physical and chemical respects, possess the very best properties which good arable soils must have, that in so far as the supply of plant nutrients is concerned they are to be classed with the most fertile, and that under cultivation they retain their fertility a very long time, which may amount to some hundreds of years. The actual conditions of climate that have produced these soils cause the years of rich harvests to alternate with those of light yields, during which the draught upon the soil is very light. Signs of exhaustion appear first in those derived from the poorer parent rocks and formed under a more humid climate. In general, the Chernozem soils begin to show first the lack of phosphoric acid and later that of nitrogen.

#### METHODS OF SAMPLING.

In an investigation such as this the method of taking the samples to be used for analysis is extremely important. We had planned to collect samples from each of the eight precipitation-belts shown in Fig. 2. None was secured from the 32 + or the 22 to 24 inch belt. On account of the recent series of dry years, McCook, which at the time of beginning the work was regarded as in the 20 to 22 inch belt, now has to be placed along with Wauneta, which was selected as representative of the 18 to 20 inch belt.

TABLE I.  
LONGITUDE, ELEVATION AND NORMAL PRECIPITATION AT TOWNS NEAR  
WHICH THE SOIL SAMPLES WERE COLLECTED.

Stations of the U.S. Wthr. Bur.	Approximate longitude	Elevation feet	Normal annual precipitation, inches	Length of record, years
Wauneta .....	101° 30'	2934	18.55	25
McCook .....	100° 40'	2506	18.83	30
Holdrege .....	99° 20'	2324	24.24	20
Hastings .....	98° 20'	1932	26.87	22
Lincoln .....	96° 40'	1189	27.51	31
Weeping Water.	96° 10'	1080	30.19	37

<sup>1</sup> See footnote 2 to Table IV.

The soil samples were collected from only virgin prairie fields near one or other of the six stations of the United States Weather Bureau mentioned in Table I, for each of which a precipitation record of twenty years or more is available. The location of these is shown in Fig. 2.

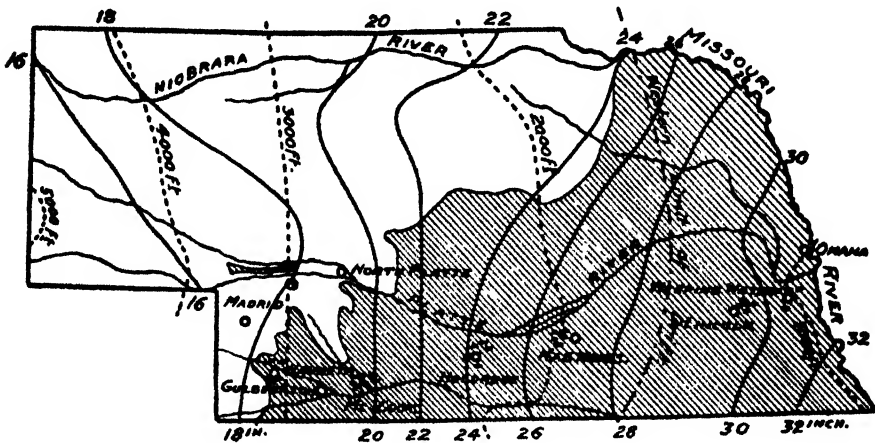


Fig. 2.—Map of Nebraska showing distribution of the loess, precipitation belts, the altitude and the location of fields sampled

The original intention was to select in each locality five level, virgin fields located to one another as are the four corners and the center of a square whose sides are two miles in length. However, on account of the great scarcity of virgin fields which were at all level, the original plan could not be adhered to closely. Along the different river courses and in the southeastern counties, where the loess overlies the Kansan till, it has been extensively eroded, in the latter in many places remaining as only small isolated plains. Also in many places on the divides in the western portion it has been eroded, and in such cases, while not usually exposing the underlying geological formation, the surface coating with its high content of organic matter, characteristic of the level or rolling prairies, has been largely removed. Thus, while numerous virgin fields were found, only a few of these were representative of the tillable areas of loess soil. The very characteristics which we desired our typical fields to possess had appealed to the farmers and caused them early to bring such land under the plow. All fields in valleys were avoided, as were also those in which the loess was found to have a depth of less than six feet. We were, however, able to select in each of the six localities mentioned in Table I five fields which, since the advent of settlers,<sup>1</sup> have been used as pastures or as meadows, or partly for both purposes. None of these

fields was more than ten miles from the Weather Bureau station whose name is given the area for the purpose of the present discussion. The legal description of each is reported in Table II. All, at least in the parts sampled, were almost level or only slightly rolling. Those of the two eastern areas, Weeping Water and Lincoln, had been used chiefly as meadows, while those of the areas to the west had been used mainly, or altogether, as pastures. The location of the selected fields with respect to the farmsteads, together with the system of farming followed, renders it improbable that there had been any additions of potash or phosphoric acid due to the application of fertilizers of any kind or to feed from elsewhere having been given the pasturing animals while on these fields. The amount of organic matter in the surface soil may be somewhat less in the fields used as meadows than would have been the case if they had been pastured, but it is probably fully as great as though they had been exposed to periodic prairie fires, such as prevailed before the settlement of the state. So, on the whole it seems extremely probable that the soil of the fields, when the samples were taken in 1909 and 1910, was similar in composition to what it was when *truly* virgin—before the advent of settlers. A typical field in each of the areas is shown in Plates I, II and III.

From each field two sets of samples were taken—the one consisting of the *foot-samples* and the other of the *inch-samples*. For the former ten borings, at intervals of 30 feet along a line across the most level portion of the field, were made to a depth of 6 feet, and composite samples prepared of each foot section, thus giving six samples, later referred to as “field-samples,” which are not to be confused with the “area-samples,” prepared by mixing equal weights of the corresponding five “field-samples.” Two soil augers, one of 2.25 and the other of 1.5 inch diameter, were employed. The former was used for taking the samples of the surface foot, as well as for enlarging and cleaning out the hole preparatory to sampling each of the lower foot sections with the smaller auger. Great care was exercised to prevent any of the soil from nearer the surface becoming mixed with the samples from the lower levels. Thus, in addition to using augers of different sizes, carefully enlarging and cleaning the hole with the larger one before taking a section with the smaller, the auger, on being withdrawn with the attached soil, was closely examined for any material which might have come from nearer the surface, and if this was found it was removed with a knife. The aerial portions of living plants were not included with the sample, but roots and plant debris were treated as integral parts of the soil.

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<sup>1</sup> Mr. A. E. Sheldon has furnished the following approximate dates at which practically all of the Government land had been taken in the different localities: Weeping Water, 1865; Lincoln, 1870; Hastings, 1885; Holdrege, 1892; McCook, 1900; and Wauneta, 1904. He states also that settlements had been made about 1854 at Weeping Water, 1858 at Lincoln, and between 1869 and 1876 in the more westerly localities.

TABLE II.  
LOCATION OF THE FIELDS FROM WHICH THE SOIL SAMPLES WERE TAKEN.

## WAUNETA.

Field No.	Part of Section	Section	Township	Range	From 6th Principal Meridian
I	W $\frac{1}{4}$ of NW $\frac{1}{4}$	4	4	36	West
II	SE $\frac{1}{4}$ of NE $\frac{1}{4}$	23	6	36	West
III	SE $\frac{1}{4}$	10	5	36	West
IV	E $\frac{1}{4}$ of NE $\frac{1}{4}$	22	6	37	West
V	NE $\frac{1}{4}$	34	5	36	West

## McCOOK.

I	SW $\frac{1}{4}$ of SW $\frac{1}{4}$	10	3	29	West
II	NE $\frac{1}{4}$ of NE $\frac{1}{4}$	10	3	29	West
III	N $\frac{1}{4}$ of NE $\frac{1}{4}$	8	3	29	West
IV	W $\frac{1}{4}$ of SW $\frac{1}{4}$	4	3	29	West
V	N $\frac{1}{4}$ of SW $\frac{1}{4}$	8	3	29	West

## HOLDREGE.

I	N $\frac{1}{4}$ of NW $\frac{1}{4}$	33	6	18	West
II	SE $\frac{1}{4}$ of NW $\frac{1}{4}$	7	5	18	West
III	E $\frac{1}{4}$ of NE $\frac{1}{4}$	9	5	18	West
IV	N $\frac{1}{4}$ of NE $\frac{1}{4}$	33	6	18	West
V	SE $\frac{1}{4}$ of NW $\frac{1}{4}$	34	6	18	West

## HASTINGS.

I	N $\frac{1}{4}$ of NE $\frac{1}{4}$	17	7	10	West
II	SE $\frac{1}{4}$ of NE $\frac{1}{4}$	12	7	11	West
III	SE $\frac{1}{4}$ of SE $\frac{1}{4}$	16	7	10	West
IV	SE $\frac{1}{4}$ of SE $\frac{1}{4}$	4	7	10	West
V	SE $\frac{1}{4}$ of SW $\frac{1}{4}$	6	7	10	West

## LINCOLN.

I	Near center of SE $\frac{1}{4}$	20	10	7	East
II	S $\frac{1}{4}$ of NE $\frac{1}{4}$	29	10	7	East
III	E $\frac{1}{4}$ of SW $\frac{1}{4}$	27	10	7	East
IV	E $\frac{1}{4}$ of E $\frac{1}{4}$	2	10	7	East
V	W $\frac{1}{4}$ of NW $\frac{1}{4}$ of SW $\frac{1}{4}$	23	10	7	East

## WEEPING WATER.

I	SE $\frac{1}{4}$ of NW $\frac{1}{4}$	27	11	11	East
II	SW $\frac{1}{4}$ of NE $\frac{1}{4}$	26	11	11	East
III	NE $\frac{1}{4}$ of SW $\frac{1}{4}$	14	10	11	East
IV	NW $\frac{1}{4}$ of SW $\frac{1}{4}$	33	11	12	East
V	N $\frac{1}{4}$ of SE $\frac{1}{4}$	34	11	12	East

It seems highly probable that the six area-samples, each a composite from 50 individual borings, from any one area represent material originally alike, any marked differences between them being due to alterations that the material experienced since its deposition. The inch sections

were taken from the first foot only, being composites of 20 (and in the fields of the Lincoln area of 50) individual samples. They were secured by means of a brass tube  $1\frac{3}{8}$  inches in diameter provided with a wide collar 6 inches from the end. The tube was forced into the ground until the collar rested firmly on the surface. The core was forced out and then, after first removing the soil to a depth of six inches by means of a spade, the second 6-inch layer was sampled in the same manner. Each of the two cores thus obtained was subdivided into six equal lengths, the first inch section having the living vegetation trimmed off level with the surface of the soil. The area inch-samples, accordingly, are composites of 100, or 250, individual samples.

In all 648 samples were subjected to more or less complete analysis in this investigation, each of the six areas being represented by 108, consisting of 36 foot-samples and 72 inch-samples.

#### THE CLIMATE.<sup>1</sup>

The altitude of the loess-covered portion of Nebraska rises gradually from east to west; all the Weeping Water fields sampled touch the 1200 foot contour line while all those at Wauneta are from 3100 to 3400 feet above the sea level.

The gradual change in altitude from east to west is not accompanied by a corresponding change in temperature, the uniformity of which, throughout the region studied, is shown by Table III, in which are given the data for four of the stations. There is no record for Wauneta, and only a very incomplete one for Weeping Water, but conditions at these two stations differ little from those at McCook and Lincoln, respectively. The mean annual temperature is  $50.1^{\circ}$  F. at Lincoln and  $51.8^{\circ}$  at McCook. February, the coldest month, shows a mean of  $24.8^{\circ}$  at the former and  $27.7^{\circ}$  at the latter, and July, the warmest month, of  $76.4^{\circ}$  and  $77.4^{\circ}$ , respectively. In most years maximum temperatures of about  $100^{\circ}$  are recorded a few times during the warm season, July, August and the early part of September, and minimum temperatures of  $15^{\circ}$  to  $20^{\circ}$  below zero during the winter months. Occasionally temperatures as high as  $110^{\circ}$  and as low as  $30^{\circ}$  below zero occur. The season without killing frosts usually extends from the first of May to the first week in October, but these have been experienced as late as the last week in May and as early as the second week in September.

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<sup>1</sup> For the data on climate we have made use of the various publications of the United States Weather Bureau dealing with Nebraska, especially the Summaries of the Climatological Data for the United States for Sections 35, 36 and 37, the Annual Summaries for the Nebraska Section, and the Annual Reports of the Chief of United States Weather Bureau. In addition, unpublished data have been furnished us by Mr. G. A. Loveland, Director of the Nebraska Section of the Bureau.

TABLE III.

TEMPERATURE DATA, IN DEGREES FAHRENHEIT, FOR THE DIFFERENT STATIONS.

## MEAN TEMPERATURES.

Station	Length of Record, Yrs. <sup>1</sup>	January	February	March	April	May	June	July	August	September	October	November	December	Annual
McCook .....	14	27.8	27.7	39.0	51.3	62.6	72.5	77.4	76.5	66.6	53.4	38.7	28.0	51.8
Holdrege .....	20	26.0	26.7	37.2	50.9	60.9	71.9	76.6	75.2	67.2	52.8	38.5	28.5	51.0
Hastings .....	22	24.4	24.7	36.9	50.1	60.6	71.5	75.6	74.4	65.3	52.8	38.8	26.8	50.0
Lincoln .....	31	21.2	24.8	36.0	50.7	62.9	71.6	76.4	74.3	65.2	53.3	38.0	26.9	50.1

## HIGHEST RECORDED TEMPERATURES.

McCook .....	14	78	74	91	98	101	106	110	107	102	94	85	71	110
Holdrege .....	20	70	78	92	101	102	106	108	108	115	92	88	65	115
Hastings .....	22	70	71	90	93	96	103	108	104	101	91	78	66	108
Lincoln .....	31	66	79	91	97	98	103	110	107	103	92	80	71	110

## LOWEST RECORDED TEMPERATURES.

McCook .....	14	*30	*38	*5	18	14	44	44	40	22	12	*8	*21	*38
Holdrege .....	20	*26	*43	*9	10	19	38	42	42	29	7	*8	*12	*43
Hastings .....	22	*30	*30	*5	18	22	41	48	43	28	9	*5	*13	*30
Lincoln .....	31	*29	*26	*11	17	25	43	49	43	27	15	*15	*18	*29

<sup>1</sup> Including 1914.

\* Below zero.

The precipitation (Table IV) decreases from east to west, the mean annual amount being a little more than 30 inches at Weeping Water and a little less than 19 at Wauneta, or an average decrease of about one inch for each 25 miles. Most of it falls during the growing season, and only less than one-tenth of it during the three winter months. June is the month of maximum precipitation and January of minimum. About half of the rainfall of May, June and July is from rains of one inch or more in 24 hours. In most years some part of the region has a storm with a rainfall exceeding 2 inches in 24 hours and occasionally this rises to 5 or even 8 inches. The fall of such a large part of the total precipitation in the form of these storms of brief duration accounts largely for the observed deficiencies of moisture for crops in seasons when the recorded rainfall would indicate an abundant supply, much of the water running off the surface before there is time for it to be absorbed by the soil. The number of days with a precipitation of 0.01 inch or more decreases from east to west more rapidly than does the total precipitation, averaging over 80 at Weeping Water and Lincoln and less than 50 at Holdrege and McCook. For those days on which the precipitation amounts to 0.01 inch or more it averages 0.34 inch at Weeping Water, 0.32 at Lincoln, 0.40 at Hastings, 0.52 at Holdrege, 0.43 at McCook, and 0.36 at Wauneta.

TABLE IV.

PRECIPITATION DATA, IN INCHES, FOR THE DIFFERENT STATIONS.

## AVERAGE PRECIPITATION.

Station	Length of Record, Yrs.	January	February	March	April	May	June	July	August	September	October	November	December	Annual
Wauneta <sup>1</sup>	25	0.26	0.69	1.03	2.04	2.54	3.34	2.47	2.74	1.35	1.13	0.39	0.57	18.55
McCook <sup>2</sup>	14	0.21	0.62	0.73	1.89	2.82	3.29	3.09	2.55	1.72	1.03	0.56	0.57	19.08
Holdrege	20	0.41	0.90	1.02	2.77	4.17	3.71	3.19	3.08	2.07	1.59	0.63	0.70	24.24
Hastings	22	0.44	1.05	1.13	2.77	3.64	4.24	3.62	3.59	2.73	2.02	0.80	0.83	26.87
Lincoln	31	0.62	0.70	1.33	2.77	4.25	4.32	3.83	3.71	2.64	1.82	0.85	0.67	27.51
W. Water	37	0.89	1.04	1.40	2.49	4.19	4.86	3.71	3.88	3.14	2.34	1.24	1.01	30.19

## HIGHEST PRECIPITATION RECORDED SINCE 1894.

Wauneta	20	0.80	2.00	3.50	4.05	5.50	7.16	9.38	5.78	3.80	3.90	2.15	3.25	32.24
McCook	20	0.70	2.32	2.85	4.96	6.87	5.63	10.86	4.60	4.53	4.65	2.02	3.19	33.97
Holdrege	20	0.90	2.20	4.25	7.90	12.36	11.83	7.15	6.19	5.05	4.35	2.58	4.19	40.21
Hastings	20	1.25	2.50	3.02	9.26	10.92	7.91	10.62	9.86	6.87	5.82	3.32	4.93	39.01
Lincoln	20	1.15	2.13	3.67	5.11	10.72	11.24	11.35	14.21	7.60	3.62	7.14	4.03	41.22
W. Water	20	2.16	2.83	3.62	4.42	11.45	12.24	10.26	10.00	9.70	3.91	9.20	3.96	41.09

## LOWEST PRECIPITATION RECORDED SINCE 1894.

Wauneta	20	0	0	0	0.12	0.20	0.65	0.73	0.30	0	0	0	0	13.13
McCook	20	0	0	0	0.05	0	0.66	0.40	0.36	0.20	T	0	0	9.34
Holdrege	20	T	T	0	T	0.30	0.85	0.50	0.95	0.25	0	0	0	16.26
Hastings	20	T	T	0.16	0.36	0.64	0.38	0.55	0.79	0.60	T	0.04	T	18.81
Lincoln	20	0.07	0.07	0.10	0.02	0.96	0.56	1.05	0.31	0.39	0.05	0.03	0.02	16.38
W. Water	20	0.10	0.10	0.10	0.18	0.55	0.39	0.73	1.25	0.38	0.11	T	0.09	21.06

## AVERAGE NUMBER OF DAYS WITH .01 INCH OR MORE OF PRECIPITATION.

Wauneta	12	1	3	3	5	7	8	6	6	4	3	2	3	51
McCook	20	2	3	3	5	5	7	5	6	3	2	1	2	44
Holdrege	23	2	3	2	4	6	7	6	5	4	3	2	3	47
Hastings	20	3	4	4	7	9	10	7	7	5	4	2	3	65
Lincoln	27	4	5	7	8	12	10	9	9	7	6	4	5	86
W. Water	28	5	6	7	9	11	11	8	8	7	6	3	4	85

<sup>1</sup> Data previous to November 1, 1901, from Ough, 10 miles north of Wauneta.<sup>2</sup> The mean for McCook for 30 years, using the record from 1882 to 1890 at Red Willow and that from 1892 to 1895 at Indianola, is 18.83 inches. The monthly means are all very similar to those here given.

The drouth frequency during the crop-growing season increases quite uniformly from east to west. Defining a *drouth period* as 30 consecutive days or more in which precipitation to the amount of 0.25 inch does not occur, the United States Weather Bureau has recently shown that the total number of drouth periods between March 1 and September 30 for the 20-year period, 1895 to 1914, inclusive, is 15 at Lincoln and 30 near the western edge of the loess.<sup>1</sup>

<sup>1</sup> Chart in National Weather and Crop Bulletin, May 5, 1915.

TABLE V.  
RELATION OF THE ANNUAL PRECIPITATION, YEAR BY YEAR, TO THE  
NORMAL (= 100).

Station	Wauneta <sup>1</sup>	McCook	Holdrege	Hastings	Lincoln	W. Water
Normal, in inches	18.55	18.83	24.24	26.87	27.51	30.19
1895.....	90	<sup>a</sup> 98	88	90	60	70
1896.....	71	107	124	127	138	128
1897.....	113	109	119	125	93	77
1898.....	113	97	75	93	102	89
1899.....	66	73	<sup>a</sup> 114	70	82	101
1900.....	81	75	<sup>a</sup> 104	103	123	114
1901.....	104	105	92	85	80	83
1902.....	...	135	<sup>a</sup> 155	145	150	135
1903.....	...	118	<sup>a</sup> 146	137	126	106
1904.....	134	112	<sup>a</sup> 101	85	101	81
1905.....	174	178	166	137	129	118
1906.....	130	108	127	87	124	79
1907.....	109	101	94	83	99	103
1908.....	134	<sup>a</sup> 95	113	119	130	122
1909.....	99	..	90	90	126	136
1910.....	76	49	77	84	114	79
1911.....	101	64	90	89	89	84
1912.....	108	77	73	95	81	89
1913.....	86	..	83	89	95	108
1914.....	93	96	67	86	145	89

<sup>1</sup> The record previous to November 1, 1901, kept at Ough, 10 miles south of Wauneta.

<sup>2</sup> For Bartley, 17 miles to the east.

<sup>3</sup> Datum for February from Culbertson, 12 miles to the west.

<sup>4</sup> Data for June and August from Kearney, 24 miles northeast.

<sup>5</sup> Datum for March from Kearney, 24 miles northeast.

<sup>6</sup> Datum for September from Kearney, 24 miles northeast.

<sup>7</sup> Data for March and November from Kearney, 24 miles northeast.

<sup>8</sup> Datum for June from Kearney, 24 miles northeast.

The relation of the annual precipitation to the normal at the different stations since 1894 is shown in Table V. The data for the years previous to 1895 are, in the case of so many of the stations, either missing or so incomplete that they do not permit of comparisons. The greatest departures shown are +78 per cent at McCook in 1905, and -40 per cent at Lincoln in 1895. Neither the greatest departures from the normal, nor the relative frequency of the years with an excess or a deficiency of precipitation (Table VI) shows any distinct relation to the longitude.

The average annual snowfall is a little less than 24 inches, it being about one inch heavier in the west than in the east. "As a rule snow covers the ground but a few days at a time after each snow storm, and the ground is covered with snow less than half of the time even during the months of the heaviest snowfall."<sup>1</sup> Much of the snow is swept by

<sup>1</sup> Loveland, G. A., Summary of Climatological Data for the United States, Sec. 37, Southern Nebraska, p. 1.



high winds into the depressions, and so contributes but little to the supply of soil moisture of the land upon which it falls. The snowfall exerts little effect upon the leaching of the soil, although agriculturally, as in the wintering of fall-sown grains, it may be very important.

TABLE VI.

THE RELATIVE FREQUENCY OF THE YEARS WITH PRECIPITATION BELOW OR ABOVE NORMAL.

Per Cent . of Normal	Wauneta %	McCook %	Holdrege %	Hastings %	Lincoln %	W.Water %
Below 71 .....	6	12	5	5	5	5
71 to 90 .....	28	16	35	50	20	43
91 to 110 .....	33	48	20	15	25	20
111 to 130 .....	16	12	25	15	35	20
131 to 150 .....	11	6	5	15	15	10
Above 150 .....	6	6	10	0	0	0

The prevailing winds are from the south or southeast during the growing season and from the northwest or north during the rest of the year. An accurate record of the velocity has been kept at only one of the six stations mentioned in Table I, viz. Lincoln. However, records are available for two other stations, Omaha and North Platte, both in the loess region, the former being about 30 miles northeast of Weeping Water and with a similar normal annual precipitation (30.66 inches) and the latter 65 miles north of McCook with a normal precipitation of 18.86 inches. At Omaha the anemometer is placed at an elevation of 121 feet, at Lincoln of 84 and at North Platte of 51. At the last the station is located in the valley of the Platte River and for this reason the velocity may be considerably lower than on the surrounding plains. An experimental substation of the University of Nebraska is situated about four miles south of North Platte and here, since 1907, a very complete meteorological record has been kept, the instruments being placed on the exposed plain at an elevation of 2985 feet, about 108 feet above the United States Weather Bureau station in the valley. Here the anemometer is two feet above the surface of the ground. The record for six months, April to September, inclusive, for the years 1908 to 1914, which has been furnished us by Mr. W. P. Snyder, has been compared with that from the United States Weather Bureau station for the same months. There are slight differences from month to month, but the average difference is very small, it being about 0.2 miles higher at the experimental substation. Accordingly we may safely assume that the data for North Platte reported in Table VII correctly represent the wind velocity near the surface of the ground on the western plains. While the average velocity recorded is 11 miles per hour at Lincoln, 10 at North Platte and 9 at Omaha, it seems probable that near the surface it would at present be

much lower at Lincoln and Omaha, especially on account of the large number of planted trees now growing. However, there appears to be no evidence that the wind velocity near the ground was not comparatively uniform throughout the region under discussion previous to the advent of settlers.

TABLE VII.  
AVERAGE HOURLY WIND MOVEMENT (IN MILES PER HOUR).

Station	Length of Record, Yrs. <sup>1</sup>	January	February	March	April	May	June	July	August	September	October	November	December	Annual
North Platte .....	40	9	9	11	12	12	10	9	9	9	9	9	8	10
Lincoln .....	20	11	11	13	14	12	10	9	9	10	11	11	10	11
Omaha .....	43	9	9	10	11	9	8	7	7	8	8	9	9	9

<sup>1</sup> Including 1914.

Data on the relative humidity are available for North Platte, Lincoln and Omaha (Table VIII). The normal is very similar at all three stations, being 71, 70 and 69 per cent, respectively, while the mean at 8 a. m. is 83, 80 and 78 per cent, and that at 8 p. m., 58, 60 and 61 per cent. Occasionally the humidity during the afternoon in summer falls below 10 per cent in the west and 20 per cent in the east. Thus the data show a very slightly greater humidity in the western part of the region than in the eastern. It is of interest in this connection that according to popular opinion in Nebraska the air is very much drier in the western part.

TABLE VIII.  
THE MEAN RELATIVE HUMIDITY.

Station	Length of Record, Yrs. <sup>1</sup>	Hour of Observation	January	February	March	April	May	June	July	August	September	October	November	December	Annual
North Platte	27	8 A.M.	83	84	83	80	83	84	85	88	85	83	79	81	83
		8 P.M.	69	67	60	51	54	56	54	55	52	54	57	65	58
		Avg.	76	76	72	66	69	70	70	72	68	69	68	73	71
Lincoln	18	8 A.M.	80	83	80	75	79	81	80	84	81	79	77	80	80
		8 P.M.	67	68	61	52	57	57	55	58	56	58	62	68	60
		Avg.	73	75	70	63	68	69	67	71	68	68	69	74	70
Omaha	27	8 A.M.	81	81	78	73	75	78	77	79	80	76	77	81	78
		8 P.M.	71	69	63	53	55	56	55	57	59	55	62	70	61
		Avg.	76	75	71	63	65	67	66	68	69	65	70	75	69

<sup>1</sup> Including 1914.

The data on the relative insolation are rather too limited to permit of definite deductions. The number of cloudy days is much greater in the east than in the west (Table IX), while the available data show much less difference in the total number of hours of sunshine (Table X), a rather surprising condition. At North Platte it averages 9 per cent more than at Omaha and 3 per cent more than at Lincoln. In this respect Lincoln resembles North Platte more than it does Omaha.

TABLE IX.

AVERAGE NUMBER OF CLEAR, PARTLY CLOUDY AND CLOUDY DAYS FOR THE SEVEN YEARS, 1908 TO 1914.

Station	Clear Days	Partly Cloudy Days	Cloudy Days
North Platte .....	175	115	75
Lincoln .....	140	111	114
Omaha .....	132	115	118

TABLE X.

PERCENTAGE OF POSSIBLE NUMBER OF HOURS OF SUNSHINE FOR THE SEVEN YEARS, 1908 TO 1914.

	North Platte %	Lincoln %	Omaha %
January .....	64	55	51
February .....	65	58	54
March .....	69	68	58
April .....	67	66	59
May .....	64	66	60
June .....	74	73	66
July .....	78	76	70
August .....	73	74	66
September .....	68	63	59
October .....	66	61	61
November .....	64	60	53
December .....	58	56	52
Year .....	68	65	59

The data on the rate of evaporation from a water surface are scanty. Russell (25, p. 10; 26, p. 558) by applying a formula to observed meteorological conditions calculated the evaporation for the entire year to be 38 or 40 inches in the extreme eastern part of Nebraska, 50 inches in the western and possibly 60 inches in the extreme southwestern corner. These are to be regarded as only rough approximations of the correct values. For Lincoln there is a record for the months of April to October from 1899 to 1909, the average for the six months, April to September inclusive, being 34.8 inches (21). As the average mean temperature, relative humidity and wind velocity for these eleven years are very simi-

lar to the normals based upon the entire record since observations were begun, it is safe to assume that this represents the normal evaporation. At the North Platte substation a record has been kept beginning with 1907. The average for the six months is 45.06 inches. As during this eight-year period both the average mean relative humidity and the average wind velocity have been lower than normal it is not possible to decide just how closely the average for these years represents the actual normal at North Platte, but probably it does not depart widely. The data are reported in Table XI. The two totals 45.06 and 35.93 inches, it should be observed, are not for the entire year, the measurements being made for only the months in which there is little freezing. The evaporation tanks were placed so that the water surface was kept at the level of the ground. The tank at Lincoln was 3 feet square and 10 inches deep, (21, p. 193) and that at North Platte 6 or 8 feet in diameter and 2 feet deep (9, p. 382). The one at Lincoln may have been somewhat protected, by neighboring buildings and trees, from the full sweep of the south and southwest winds, but on the whole the conditions were such as to make the records satisfactorily comparable.

TABLE XI.  
EVAPORATION FROM A FREE WATER SURFACE AT NORTH PLATTE  
AND LINCOLN.

	April Inches	May Inches	June Inches	July Inches	Aug. Inches	Sept. Inches	Tl. 6 mos. Inches
No. Platte, 1907 to 1914	5.92	6.78	8.54	9.00	8.41	6.41	45.06
Lincoln, 1895 to 1910..	4.71	5.85	6.57	7.57	6.39	4.84	35.93

Bigelow (8, p. 5) reports the annual evaporation at Dutch Flats at the western edge of the state, to be 65.67 inches for the years 1909-1910, using a tank 4 feet in diameter. As observations were not made for the winter months the rates for these were found by interpolation.

The relative evaporation from a free-water surface depends upon the intensity of the solar radiation and the cloudiness, as well as upon the temperature, the relative humidity of the air and the wind velocity. Throughout the region under consideration the last three factors are found to be very uniform, while the first also is to be regarded as uniform, but the cloudiness decreases as we proceed from east to west. To this difference in the amount of sunshine we must attribute the observed differences in the rate of evaporation. Although there are no records of the evaporation in the central portion, represented by Hastings and Holdrege, it seems safe to assume that there it is intermediate between that at Lincoln and that at North Platte, while that at both McCook and Wauneta may be considered very similar to that at North Platte.

To summarize the climatic relations we may state that as we proceed from east to west there is experienced a gradual decrease in the total precipitation and in the cloudiness, with an increase in the rate of evaporation from a water surface and in the frequency of drouths, while the distribution of rainfall and snowfall, the temperature conditions, the wind velocity and the relative humidity remain quite uniform.

#### HYGROSCOPICITY.

As the variations in the hygroscopicity of soils are due to variations in texture a determination of the former serves to indicate the uniformity in texture of a series of samples. This single-valued expression of the relative heaviness of soils was suggested by Hilgard in 1860 (15, p. xi). The simple method of determining this value which he later designates the "hygroscopic coefficient" (16, p. 16; 17, p. 17)—the percentage of water absorbed by a dry soil from a saturated atmosphere—probably serves quite as well as the more complicated and time-consuming method later developed by Rodewald and Mitscherlich (24). Briggs and McLane (10; 11, p. 140) have introduced a somewhat similar method of expressing the relative texture of soils as a single factor—the "moisture equivalent," defined as the "maximum percentage of moisture a soil can retain in opposition to a centrifugal force equal to 1000 times the force of gravity." Briggs and Shantz (12, p. 64) have concluded that this may serve as an indirect method for the determination of the hygroscopic coefficient, the latter being 0.37 times the moisture equivalent. This method may have some advantages in convenience of execution, the absence of any need of a constant-temperature room, the lesser skill required on the part of the operator and a somewhat closer concordance of duplicate determinations, but these may be in many cases more or less completely offset by the cost of the apparatus required and the difficulties in installation. Further the moisture equivalent in itself expresses only the relative texture while the hygroscopic co-efficient does this quite as well, and at the same time indicates the lower limit of moisture available for the support of life of other than strictly xerophytic plants (1).

The data reported in Tables XII and XIII are the averages of concordant duplicate determinations made by exposing the air-dried soil in a layer *ca* 1 mm. in thickness to a saturated atmosphere for 24 hours, the temperature of the air not varying more than 1°C during the period.

In Table XII there are reported the hygroscopic coefficients of the foot sections from each of the thirty fields, and in Table XIII the averages for the foot levels of the different areas, each value in the latter table thus representing a composite sample from 50 borings and also the average of 10 determinations. In Table XIV are reported the averages of the coefficients for the six-foot sections from the different fields, each value here representing a composite of 60 individual samples and the average of 12 determinations.

TABLE XII.  
HYGROSCOPIC COEFFICIENTS OF THE FOOT SECTIONS FROM THE FIVE FIELDS  
OF EACH AREA.

## WAUNETA.

Depth Ft.	Field I	Field II	Field III	Field IV	Field V	Average
1	9.9	9.0	8.7	7.9	9.8	9.1
2	10.3	9.3	9.2	9.0	10.2	9.6
3	10.8	9.6	8.9	9.4	9.7	9.7
4	10.7	10.3	9.5	9.4	9.7	9.9
5	9.7	9.0	9.9	7.8	8.8	9.0
6	8.8	7.6	9.7	6.9	8.4	8.3
Average	10.0	9.1	9.3	8.4	9.4	9.2

## McCOOK.

1	10.6	9.6	10.0	10.3	9.6	10.0
2	11.6	11.0	10.1	11.2	10.8	10.9
3	10.9	11.8	9.8	10.7	10.1	10.7
4	9.5	10.5	8.9	10.2	9.3	9.7
5	9.0	10.3	8.5	9.1	8.7	9.1
6	9.6	10.4	8.2	8.9	8.5	9.1
Average	10.3	10.6	9.2	10.1	9.5	9.9

## HOLDREGE.

1	10.6	10.0	9.5	10.4	9.9	10.1
2	11.3	11.0	10.3	11.9	11.5	11.2
3	10.8	11.0	11.7	11.4	11.8	11.3
4	9.6	10.1	10.2	10.0	10.9	10.2
5	8.8	9.1	9.7	9.3	10.8	9.5
6	8.5	9.0	9.5	8.8	11.0	9.4
Average	10.0	10.0	10.1	10.3	11.0	10.3

## HASTINGS.

1	9.4	9.1	10.3	9.1	10.0	9.6
2	11.4	11.2	11.4	12.0	11.9	11.6
3	12.8	11.7	12.0	13.3	12.4	12.4
4	11.1	11.2	11.0	11.6	10.5	11.1
5	10.7	10.4	10.5	11.3	10.6	10.7
6	10.8	10.1	10.4	11.1	11.0	10.7
Average	10.9	10.6	10.9	11.4	11.1	11.0

## LINCOLN.

1	10.9	12.2	11.7	13.4	11.7	12.0
2	14.4	15.1	13.8	15.2	13.5	14.4
3	14.3	14.0	12.5	14.1	13.1	13.6
4	13.6	13.2	12.2	13.9	12.7	13.1
5	13.2	13.1	12.2	12.5	12.6	12.7
6	12.8	13.0	12.3	12.4	12.7	12.6
Average	13.2	13.4	12.5	13.6	12.7	13.1

## WEEPING WATER.

1	12.0	12.4	12.6	12.0	11.6	12.1
2	13.3	13.7	14.4	13.4	13.5	13.7
3	14.4	13.8	14.1	13.6	13.9	14.0
4	12.8	13.1	13.8	12.6	12.8	13.0
5	12.6	12.5	13.4	12.5	12.2	12.6
6	12.3	12.4	13.2	12.6	12.2	12.5
Average	12.9	13.0	13.6	12.8	12.7	13.0

TABLE XIII.

HYGROSCOPIC COEFFICIENTS OF THE FOOT SECTIONS FROM THE DIFFERENT AREAS.

Depth Ft.	Wauneta	McCook	Holdrege	Hastings	Lincoln	Wpg.Wtr.	Average
1	9.1	10.0	10.1	9.6	12.0	12.1	10.5
2	9.6	10.9	11.2	11.6	14.4	13.7	11.9
3	9.7	10.7	11.3	12.4	13.6	14.0	11.9
4	9.9	9.7	10.2	11.1	13.1	13.0	11.1
5	9.0	9.1	9.5	10.7	12.7	12.6	10.6
6	8.3	9.1	9.4	10.7	12.6	12.5	10.5
Average	9.2	9.9	10.3	11.0	13.1	13.0	11.1

The hygroscopicity is, on the whole, strikingly uniform, both from field to field in any one area and from the surface downward in the same field. It is lowest in the two western areas, in the fields of which it is similar, and highest in the two eastern in which also it is similar. Considering the six depths from the individual fields it is seen to show a maximum in either the second or third foot in 28 of the fields, while in the other two—II and III at Wauneta—it is higher in the fourth foot, but by less than 1 per cent. Comparing the values for these two feet it will be seen that there is no regularity, the maximum being shown in the second foot of all the Lincoln field, and in the third foot of all those at Hastings, while in each of the four other areas some fields show the maximum in the second and others in the third foot. The average for all thirty fields is 11.9 for both the second and the third foot. The values for the fifth foot are in general practically the same as for the sixth. The minimum value in the three eastern areas is found in the first foot, and in the three western in the sixth.

TABLE XIV.

AVERAGE HYGROSCOPIC COEFFICIENTS FOR THE DIFFERENT FIELDS.

Field No.	Wauneta	McCook	Holdrege	Hastings	Lincoln	Wpg.Wtr.
I	10.0	10.3	10.0	10.9	13.2	12.9
II	9.1	10.6	10.0	10.6	13.4	13.0
III	9.3	9.2	10.1	10.9	12.5	13.6
IV	8.4	10.1	10.3	11.4	13.6	12.8
V	9.4	9.5	11.0	11.1	12.7	12.7
Average	9.2	9.9	10.3	11.0	13.1	13.0

If we compare the averages for the five fields (Table XIV) it will be seen that those for Weeping Water are similar to those for Lincoln, the maximum value for the ten fields being 13.6 and the minimum 12.5; the Hastings fields all show lower values, from 10.6 to 11.4, while those for Holdrege, from 10.0 to 11.0, are similar; the fields at McCook, 9.2 to 10.6, and at Wauneta, 8.4 to 10.0, have averages which are slightly lower,

but three of the McCook and one of the Wauneta fields show values practically the same as four of those at Holdrege. The uniformity may be well illustrated by pointing out that, in estimating the free moisture in the first six feet of soil in any one of the ten fields in the two eastern areas, it would give entirely satisfactory results to employ the average value, 13.0, instead of using the values actually found for the different fields. For those at Hastings we could use 11.0, and for those at Holdrege and McCook, 10.0. The maximum difference between two fields in the same area is shown at Wauneta where Field IV, which is at the actual border of the loess (Plate III, Fig. 1), the samples being collected a quarter of a mile from the edge, shows a lower value than any other field.

The uniformity in texture of the loess is illustrated by the data in Table XV from a single ranch near Madrid, the samples being taken from a type of soil which has later been mapped by the Bureau of Soils of the United States Department of Agriculture as Sidney Silt Loam, "the weathered product of one of the late Tertiary deposits."<sup>1</sup>

TABLE XV.  
HYGROSCOPIC COEFFICIENTS OF TEN SETS OF SOIL SAMPLES FROM A SINGLE RANCH NEAR MADRID, NEBRASKA.

Depth Ft.	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10
1	7.8	8.5	8.0	7.5	2.0	5.9	8.5	7.0	5.3	1.9
2	10.4	9.8	9.8	10.5	2.8	6.3	10.2	7.8	5.1	1.8
3	10.2	9.8	11.3	9.2	2.5	6.4	12.4	9.3	3.3	1.7
4	7.0	8.3	7.7	6.9	4.5	7.1	13.1	13.0	3.0	1.5
5	7.0	6.9	6.4	6.6	5.9	7.7	12.2	14.2	3.0	1.8
6	7.8	7.4	6.3	6.9	6.7	9.3	9.0	12.8	1.9	1.9

The proportion of organic matter in the surface foot is highest in the fields of the eastern areas, those in which, as above mentioned, the hygroscopic coefficient shows a minimum in the surface foot. This would suggest that the organic matter exerts little, if any, influence in increasing the hygroscopicity. This view is confirmed by the data presented in Table XVI, giving the hygroscopic coefficients for the different inch sections from each of the areas. From Table XXIX below it will be seen that the organic matter decreases rapidly from the surface downward, the range exceeding 100 per cent for each of the areas. The hygroscopic coefficient, on the other hand varies but little and tends to show an increase, rather than a decrease, from the surface downward. Either the organic matter exerts no effect upon the hygroscopicity, or, as seems more probable, the soil of the first foot, as we proceed from the surface downward, increases in fineness of texture to such an extent that it more than counterbalances the influence of the decrease in organic matter.

<sup>1</sup> Bur. Soils—1913—Reconnaissance Survey of Western Nebraska, U. S. Dept. Agr., Bur. Soils, Field Operations of 1911.



TABLE XVI.

HYGROSCOPIC COEFFICIENTS OF THE DIFFERENT INCH SECTIONS FROM THE DIFFERENT AREAS. EACH IS A COMPOSITE OF 100 OR MORE INDIVIDUAL SAMPLES.

Depth In.	Wauneta	McCook	Holdrege	Hastings	Lincoln	Wpg. Wtr.	Average
1	8.5	8.5	10.9	10.9	11.5	11.5	10.3
2	8.2	8.3	10.3	9.7	11.2	11.0	9.8
3	8.2	8.4	9.9	8.9	11.0	11.0	9.6
4	8.3	8.3	9.5	8.5	11.1	11.1	9.5
5	8.2	8.7	9.4	8.3	11.4	11.2	9.5
6	8.6	9.3	9.4	9.0	11.8	11.2	9.9
7	8.7	9.5	9.7	9.5	11.9	11.5	10.1
8	8.8	9.8	9.9	9.5	12.1	12.1	10.4
9	8.6	9.9	10.0	9.5	13.0	12.3	10.6
10	8.8	10.3	10.9	9.7	12.6	12.6	10.8
11	9.0	10.3	10.2	10.0	12.9	12.5	10.8
12	8.7	10.2	10.2	10.2	13.1	12.8	10.9
Average	8.6	9.3	10.0	9.5	12.0	11.7	10.2

If the hygroscopicity is but little influenced by an increase in the organic matter of the soil while the water-holding capacity is distinctly increased, the effect upon crop growth of this increase will be much greater than would be expected from the relative change in the total water content, as the increase will be confined to the available portion of the soil moisture.

TABLE XVII.

HYGROSCOPIC COEFFICIENTS OF THE DIFFERENT INCH SECTIONS FROM THE FIVE FIELDS NEAR LINCOLN.

Depth In.	Field I	Field II	Field III	Field IV	Field V	Average
1	11.2	10.5	10.9	12.2	11.5	11.3
2	9.3	10.6	11.8	12.5	11.1	11.1
3	8.9	10.7	11.3	12.2	11.1	10.8
4	8.9	11.1	11.7	13.0	11.1	11.2
5	9.2	11.3	11.4	12.6	11.5	11.2
6	9.3	11.4	11.6	12.6	11.8	11.3
7	9.3	11.6	12.1	13.3	11.9	11.6
8	9.5	11.8	12.1	13.6	11.9	11.8
9	9.4	12.5	12.6	14.1	11.8	12.1
10	10.0	12.8	13.2	14.5	12.0	12.5
11	10.0	13.9	13.2	15.1	12.4	12.9
12	10.1	14.7	13.9	14.6	13.6	13.4
Average	9.6	11.9	12.2	13.4	11.8	11.8

Only in the case of the Lincoln area were the field inch-sections subjected to the determination of the hygroscopic coefficient. (Table XVII). It will be seen that there is quite as much difference between the sections from Fields I and IV as between the two most dissimilar sets of area-samples. In these also there is to be found no connection between the relative amounts of organic matter and the hygroscopicity (Table XXVII). The organic carbon in the inch-sections from the individual

fields was not determined but the nitrogen was, and as will be shown below the latter bears an almost constant relation to the former. The inch-samples from Field I show the highest content of nitrogen (Table XXI) but the lowest hygroscopic coefficients, while Field IV in which the latter values are highest is the one next to the lowest in nitrogen—additional evidence of the slight influence of the organic matter upon the hygroscopicity. However, the high hygroscopic coefficients for peat soils, containing from 80 to 95 per cent organic matter, which we have found to be from 50 to 60, indicate that the organic matter should be expected to exert at least a slight effect in the case of the prairie soils.

#### NITROGEN.

Nitrogen was determined in all the field foot-samples (Table XVIII) and field inch-samples (Table XXI), the average of these furnishing the data for the area samples (Tables XIX and XXII). The average nitrogen content for the six feet of each field is given in Table XX.

In all the fields the nitrogen, as was to be expected, decreases from the surface downward. The few cases in which it is found slightly lower in the fourth or fifth foot than in the sixth, as in Wauneta III and McCook I, may safely be attributed to the experimental error of sampling or of analysis. It decreases from east to west. According to the amount in the surface foot, the fields fall into three groups: one with .125 to .146, another with .164 to .209, and the third with .228 to .245 per cent. All the Wauneta and McCook fields are in the first, those at Holdrege and Hastings in the second and those at Lincoln and Weeping Water in the third. This separation into three groups holds also when we consider the averages of the inch-sections from each field (Table XXI), a distinct set of samples.

On the basis of the nitrogen content of the second foot two groups are recognizable, the one including all the fields of the two eastern areas with .078 to .111 and the other those of the four western with .122 to .171 per cent. While the first foot of the Lincoln fields is similar in nitrogen to that of those at Weeping Water, the second foot is lower in the former than in the latter. In the fields of these two eastern areas the nitrogen in the second foot is similar to that in the first foot of the McCook and Wauneta areas. The differences in the third and lower foot are not sufficient to permit of any grouping of areas, although the nitrogen content is in general higher in the eastern than in the western sub-soils from the same depth.

The differences between areas are not so regular when the averages for the fields are compared (Table XX), Fields II and IV at Hastings, for instance, showing a lower content than II at McCook or III at Wauneta. The differences in the nitrogen content of the first foot in the different areas might be due, partly or wholly, to differences in texture, a coarser

TABLE XVIII.  
NITROGEN IN THE FOOT SECTIONS FROM THE FIVE FIELDS OF EACH AREA.

## WAUNETA.

Depth Foot	Field I %	Field II %	Field III %	Field IV %	Field V %	Average %
1	.135	.138	.144	.129	.132	.136
2	.080	.078	.092	.082	.079	.082
3	.059	.058	.072	.078	.060	.065
4	.043	.056	.042	.047	.043	.046
5	.033	.035	.049	.039	.036	.038
6	.028	.025	.049	.023	.027	.030
Average	.063	.065	.075	.066	.063	.066

## McCOOK.

1	.143	.146	.138	.143	.125	.139
2	.079	.090	.080	.088	.085	.084
3	.048	.067	.052	.052	.049	.054
4	.036	.049	.037	.036	.034	.038
5	.031	.037	.034	.029	.031	.032
6	.034	.031	.030	.029	.027	.030
Average	.062	.070	.062	.063	.059	.063

## HOLDREGE.

1	.172	.174	.164	.189	.209	.182
2	.089	.098	.111	.104	.103	.101
3	.055	.064	.074	.075	.055	.065
4	.038	.043	.053	.056	.037	.045
5	.031	.038	.040	.032	.027	.034
6	.034	.034	.039	.034	.028	.034
Average	.070	.075	.080	.082	.076	.077

## HASTINGS.

1	.171	.174	.183	.169	.174	.174
2	.095	.095	.102	.093	.104	.098
3	.059	.053	.062	.054	.059	.057
4	.043	.039	.044	.032	.046	.041
5	.032	.029	.041	.025	.040	.033
6	.029	.027	.033	.024	.034	.029
Average	.071	.069	.077	.066	.076	.072

## LINCOLN.

1	.241	.245	.234	.238	.242	.240
2	.122	.145	.124	.122	.133	.129
3	.068	.073	.072	.063	.072	.070
4	.050	.060	.058	.065	.065	.060
5	.045	.047	.043	.040	.036	.042
6	.054	.046	.042	.039	.036	.043
Average	.097	.103	.095	.094	.097	.097

## WEEPING WATER.

1	.228	.232	.242	.243	.237	.236
2	.149	.149	.146	.171	.153	.154
3	.081	.086	.080	.097	.070	.083
4	.053	.053	.052	.073	.064	.059
5	.047	.043	.041	.044	.041	.043
6	.040	.039	.035	.039	.039	.038
Average	.100	.100	.099	.111	.101	.102

TABLE XIX.  
NITROGEN IN THE FOOT SECTIONS FROM THE DIFFERENT AREAS.

Depth	Wauneta	McCook	Holdrege	Hastings	Lincoln	Wpg.Wtr.	Average
Foot	%	%	%	%	%	%	%
1	.136	.139	.182	.174	.240	.236	.185
2	.082	.084	.101	.098	.129	.154	.108
3	.065	.054	.065	.057	.070	.083	.066
4	.046	.038	.045	.041	.060	.059	.048
5	.038	.032	.034	.033	.042	.043	.037
6	.030	.030	.034	.029	.043	.038	.034
Average	.066	.063	.077	.072	.097	.102	.079

soil tending to accumulate less organic matter. However, a comparison of the hygroscopic coefficients for the first foot of the McCook and Hastings fields, makes it evident that the texture does not account for the differences between these in nitrogen; at McCook the hygroscopic coefficient averages higher, while the five fields in that area are lower in nitrogen than those at Hastings. The greater amount of nitrogen is probably due to the greater production of vegetable material, both as aerial portions and as roots, in the eastern areas, which in turn is a consequence of the greater rainfall and lower evaporation.

TABLE XX.  
AVERAGE NITROGEN CONTENT OF THE FIRST SIX FEET OF EACH FIELD IN EACH OF THE SIX AREAS.

Field No.	Wauneta %	McCook %	Holdrege %	Hastings %	Lincoln %	Wpg.Wtr. %
I	.063	.062	.070	.071	.097	.100
II	.065	.070	.075	.069	.103	.100
III	.075	.062	.080	.077	.095	.099
IV	.066	.063	.082	.066	.094	.111
V	.063	.059	.076	.076	.097	.101
Average	.066	.063	.077	.072	.097	.102

From Table XXI it may be seen that in the first foot the nitrogen in all fields is highest in the first inch, while the amount in the twelfth is approximately half of that in the first.

A fair comparison of the nitrogen content of two fields is difficult, for the reason that if the soil of one is the more compact, the sampling tools will penetrate relatively deeper and as a result the soil sample will show a lower nitrogen content. For this reason, instead of taking the samples that are to be under comparison to the same depth, they should be taken so as to secure the same dry weight of each in a section with the same surface.

To determine the influence of such differences in density upon the found nitrogen content we weighed the samples from all the fields of each of the areas, except the two first sampled—Lincoln and Holdrege. In none of the twenty fields did we find any relation between depth and

TABLE XXI.  
NITROGEN IN THE INCH SECTIONS FROM THE SURFACE FOOT OF THE FIVE  
FIELDS OF EACH AREA.

WAUNETA.

Depth Inch	Field I %	Field II %	Field III %	Field IV %	Field V %	Average %
1	.219	.202	.224	.184	.197	.205
2	.192	.183	.169	.151	.169	.173
3	.177	.170	.162	.145	.173	.165
4	.161	.146	.159	.130	.150	.149
5	.145	.131	.140	.114	.142	.134
6	.138	.120	.132	.112	.136	.128
7	.128	.114	.124	.103	.121	.118
8	.120	.108	.117	.103	.114	.112
9	.122	.109	.116	.102	.115	.113
10	.113	.100	.107	.095	.106	.104
11	.105	.093	.103	.088	.102	.098
12	.101	.088	.099	.087	.099	.095
Average	.143	.130	.138	.118	.135	.132

McCOOK.

1	.220	.208	.205	.212	.174	.204
2	.157	.184	.169	.165	.151	.161
3	.158	.173	.164	.165	.155	.161
4	.167	.163	.162	.157	.145	.151
5	.153	.150	.145	.148	.133	.141
6	.144	.145	.135	.140	.133	.134
7	.128	.132	.129	.127	.122	.121
8	.115	.120	.119	.116	.109	.111
9	.113	.116	.113	.109	.100	.110
10	.099	.112	.106	.099	.093	.101
11	.094	.104	.097	.096	.087	.091
12	.094	.101	.090	.090	.086	.091
Average	.137	.142	.136	.135	.124	.131

HOLDREGE.

1	.238	.391	.361	.318	.305	.323
2	.213	.286	.282	.261	.291	.267
3	.204	.259	.246	.221	.239	.234
4	.182	.229	.206	.200	.215	.206
5	.167	.204	.188	.179	.196	.187
6	.161	.180	.168	.167	.173	.170
7	.139	.172	.158	.155	.158	.156
8	.137	.153	.144	.147	.149	.146
9	.132	.152	.140	.141	.145	.142
10	.122	.142	.134	.135	.141	.135
11	.124	.134	.136	.132	.137	.133
12	.115	.131	.130	.127	.132	.127
Average	.161	.203	.191	.182	.190	.186

TABLE XXI.—(Continued).

## HASTINGS.

Depth Inch	Field I %	Field II %	Field III %	Field IV %	Field V %	Average %
1	.287	.312	.350	.409	.411	.354
2	.216	.235	.259	.235	.241	.237
3	.196	.203	.219	.206	.200	.205
4	.182	.184	.195	.185	.181	.186
5	.170	.176	.183	.175	.169	.175
6	.159	.164	.168	.166	.154	.162
7	.154	.161	.165	.156	.146	.156
8	.149	.155	.158	.148	.138	.150
9	.142	.147	.150	.147	.135	.144
10	.140	.142	.145	.143	.130	.140
11	.136	.138	.142	.141	.127	.137
12	.131	.134	.138	.137	.122	.132
Average	.172	.179	.189	.187	.180	.181

## LINCOLN.

Depth Inch	Field I %	Field II %	Field III %	Field IV %	Field V %	Average %
1	.495	.296	.293	.304	.348	.347
2	.327	.273	.254	.253	.287	.279
3	.299	.255	.235	.240	.265	.259
4	.275	.250	.224	.231	.248	.245
5	.262	.241	.212	.223	.231	.234
6	.253	.229	.205	.211	.215	.223
7	.224	.221	.195	.205	.205	.210
8	.219	.214	.186	.192	.196	.201
9	.217	.202	.176	.184	.184	.193
10	.202	.186	.167	.175	.173	.181
11	.197	.177	.160	.163	.166	.173
12	.185	.168	.148	.159	.154	.163
Average	.260	.226	.205	.212	.223	.225

## WEEPING WATER.

Depth Inch	Field I %	Field II %	Field III %	Field IV %	Field V %	Average %
1	.310	.281	.481	.290	.345	.311
2	.267	.266	.390	.275	.288	.297
3	.250	.248	.273	.259	.269	.260
4	.237	.235	.249	.246	.253	.244
5	.225	.230	.235	.237	.240	.233
6	.218	.219	.226	.232	.231	.225
7	.211	.213	.221	.225	.214	.217
8	.202	.209	.212	.215	.204	.208
9	.200	.201	.205	.210	.199	.203
10	.198	.199	.195	.207	.193	.198
11	.190	.185	.186	.203	.187	.190
12	.181	.183	.181	.197	.185	.185
Average	.224	.222	.255	.233	.234	.234

density farther than that, as a rule, the first and second inch sections were lighter than the deeper ones. If the average weight of the 1-3 inch section in the 20 fields be placed at 100, the average weights for the 4-6, 7-9 and 10-12 inch sections would become 111, 110 and 111, respectively. The surface foot of the eastern fields is somewhat denser than that of the western, the relative averages being: Wauneta 94, McCook 90, Hastings

TABLE XXII.  
NITROGEN IN THE INCH SECTIONS OF THE SURFACE FOOT OF THE  
SIX DIFFERENT AREAS.

Depth Inch	Wauneta %	McCook %	Holdrege %	Hastings %	Lincoln %	Wpg.Wtr. %	Average %
1	.205	.204	.323	.354	.347	.341	.296
2	.173	.165	.267	.237	.279	.297	.236
3	.165	.163	.234	.205	.259	.260	.214
4	.149	.159	.206	.186	.246	.244	.198
5	.134	.146	.187	.175	.234	.233	.185
6	.128	.139	.170	.162	.223	.225	.175
7	.118	.128	.156	.156	.210	.217	.164
8	.112	.118	.146	.150	.201	.208	.156
9	.113	.110	.142	.144	.193	.203	.151
10	.104	.102	.135	.140	.181	.198	.143
11	.098	.096	.133	.137	.173	.190	.138
12	.095	.092	.127	.132	.163	.185	.132
Average	.132	.135	.186	.181	.225	.234	.182

110, and Weeping Water 108 (Table XXIII). The greater density of the samples from the eastern fields may be due to differences in moisture content at the time of sampling, the soil of the western areas being dry and that of the eastern more or less moist. In the case of all the Wauneta and McCook fields, of II and V at Hastings, and II, III and V at Weeping Water, samples for moisture determination were taken at the same time as those for chemical analysis. In the first foot of all the fields of the western areas the free water, the difference between the total moisture and the hygroscopic coefficient, lay between 0.0 and 2.0 per cent, while in the five eastern fields mentioned it was 13.4, 9.5, 14.4, 6.8 and 11.6 per cent respectively. When moist the soil tends to permit of a greater compression as the sampling tube is forced in. The difference to be observed among the ten western fields could not have been influenced by the moisture content as this was similar in all. At Hastings all five fields were sampled within less than five days of one another during a period of fair weather following a succession of rains. At Weeping Water the fields were sampled near the end of November, after two months of almost rainless weather; here a difference of 100 per cent in the free water content was not accompanied by a distinct difference in the found density. It may be that the density of the surface foot of the western prairies is less than that of the eastern, but we do not consider our data sufficient to justify any such definite conclusion.

The depth of sampling should vary inversely as the found density. If the depths indicated in Table XXIV had been employed, the average dry weight of the twenty cores from each field used in the preparation of the inch samples would have been practically the same. From the data in Tables XXI and XXIV we can calculate the per cent of nitrogen in the first foot that would have been found if such a method of sampling had

been followed. The extent to which they differ from the averages of the twelve sections given in Table XXI is shown in Table XXV. While in general the difference is not great it is evident that in any fine work the relative weights of the samples under comparison should not be ignored. The importance of this has previously been pointed out (4, 2). It is

TABLE XXIII.

RELATIVE DENSITY OF THE SURFACE FOOT OF SOIL FROM DIFFERENT FIELDS.  
(AVERAGE OF THE 20 = 100.)

Field No.	Wauneta	McCook	Hastings	Weeping Water
I	93	102	88	101
II	99	98	125	110
III	90	81	127	111
IV	95	83	95	111
V	92	87	115	107
Average	94	90	110	108

TABLE XXIV.

DEPTH IN INCHES TO WHICH THE DIFFERENT FIELDS SHOULD HAVE BEEN  
SAMPLED IN ORDER TO SECURE THE SAME WEIGHT OF SOIL FROM EACH.

Field No.	Wauneta	McCook	Hastings	Weeping Water
I	10.5	9.5	11.0	9.5
II	10.0	10.0	8.0	9.0
III	11.0	12.0	7.5	9.0
IV	10.5	11.5	10.5	9.0
V	11.0	11.0	8.5	9.0

readily apparent that the soil of a pasture field may be expected to show a greater density than that of a meadow, and, consequently, if both are sampled to the same depth, also a lower nitrogen content, although the two may be equally rich in this constituent.

The differences above are quite similar to those shown between the nitrogen content of the composite of 10 individual samples taken with an auger and that secured by the tube from the same field (Table XXVI).

TABLE XXV.

CHANGE IN THE NITROGEN CONTENT FOUND FOR THE FIRST FOOT THAT  
WOULD HAVE BEEN CAUSED BY USING THE SAME WEIGHT, INSTEAD OF  
THE SAME DEPTH OF SOIL.

Field No.	Wauneta	McCook	Hastings	Weeping Water
I	.005	.011	.004	.009
II	.008	.008	.019	.010
III	.003	.000	.025	.021
IV	.004	.002	.007	.010
V	.003	.011	.021	.016



TABLE XXVI.

DIFFERENCES IN NITROGEN CONTENT FOUND IN THE TWO SETS OF SAMPLES FROM THE SAME FIELD. A DEFICIENCY FOR THE AVERAGE OF THE INCH SECTIONS IS INDICATED BY THE MINUS SIGN.

Field No. No.	Wauneta %	McCook %	Holdrege %	Hastings %	Lincoln %	Wpg.Wtr. %
I	.006	— .006	— .011	.001	.019	— .004
II	— .007	— .004	.029	.005	— .019	— .010
III	— .007	— .002	.027	.006	— .029	.013
IV	— .011	— .008	— .007	.018	— .026	— .010
V	.003	— .001	— .019	.006	— .019	— .001

## ORGANIC CARBON.

The organic carbon was determined by combustion with copper oxide in a current of oxygen, the 10-gram sample of soil having first been treated with phosphoric acid solution and evaporated to dryness. It was found desirable in the case of the calcareous subsoils to repeat the treatment with phosphoric acid in order to ensure the full decomposition of carbonates. Analyses were made of all the field foot-samples (Table XXVII), the averages of which give the data for the area foot-samples (Table XXVIII), and also of the area inch-samples (Table XXIX). The average carbon content for the six foot-sections from each field is given in Table XXX.

In all the areas the carbon decreases from the surface downward, both in the foot and in the inch sections. As was the case with the nitrogen content, the fields may be placed in three groups according to the amount of carbon in the surface foot. The Wauneta and McCook fields have between 1.49 and 1.81 per cent, the Holdrege and Hastings fields between 1.93 and 2.50, and those of the eastern two areas between 2.76 and 3.07. On the basis of the composition of the second foot only two distinct groups, as with the nitrogen, are recognizable. For the third, fourth, fifth and sixth feet there is no grouping, the highest average content being shown by the two outer groups, that for the four feet for Weeping Water being .44 per cent against .42 for Wauneta and that for the fourth to sixth foot being .35 and .32 per cent, respectively. The fields of the two eastern areas show the same relative differences in the carbon as in the nitrogen content, they being similar in the first but those at Lincoln having, with one exception, a distinctly smaller amount in the second foot.

Again, as with nitrogen, the differences are not so regular when we consider the average for the six feet of the different fields (Table XXX), the distinction between the western and the central areas disappearing.

The rate of decrease in the carbon content from the first to the twelfth inch in the surface foot is quite similar in all the areas.

TABLE XXVII.  
ORGANIC CARBON IN THE FOOT SECTIONS FROM THE FIVE FIELDS  
OF EACH AREA.

## WAUNETA.

Depth Foot	Field I %	Field II %	Field III %	Field IV %	Field V %	Average %
1	1.67	1.64	1.70	1.54	1.49	1.61
2	.83	.75	.91	.77	.73	.80
3	.55	.65	.67	.77	.50	.63
4	.38	.59	.51	.45	.36	.46
5	.29	.28	.50	.25	.30	.32
6	.24	.21	.46	.18	.22	.26
Average	.66	.69	.79	.66	.60	.68

## McCOOK.

1	1.63	1.81	1.66	1.72	1.41	1.65
2	.71	.92	.77	.88	.70	.80
3	.42	.72	.53	.55	.58	.56
4	.32	.45	.32	.29	.31	.34
5	.25	.35	.27	.22	.33	.28
6	.23	.22	.22	.19	.20	.21
Average	.59	.74	.63	.64	.59	.64

## HOLDREGE.

1	2.10	2.16	2.24	2.32	2.50	2.26
2	.93	1.05	1.22	1.10	1.11	1.08
3	.49	.56	.75	.69	.50	.60
4	.33	.36	.46	.47	.27	.38
5	.21	.29	.29	.21	.21	.24
6	.21	.23	.26	.19	.15	.21
Average	.71	.78	.87	.83	.79	.79

## HASTINGS.

1	2.02	1.97	2.19	1.93	2.19	2.06
2	1.04	1.01	1.14	.93	1.13	1.05
3	.64	.56	.56	.44	.67	.57
4	.36	.32	.35	.21	.42	.33
5	.23	.20	.29	.14	.33	.24
6	.16	.15	.20	.14	.25	.18
Average	.74	.70	.79	.63	.83	.74

## LINCOLN.

1	2.87	2.88	2.77	2.88	2.98	2.88
2	1.32	1.32	1.19	1.31	1.46	1.32
3	.66	.62	.61	.63	.77	.66
4	.34	.36	.34	.31	.38	.35
5	.22	.25	.28	.23	.27	.25
6	.19	.23	.22	.25	.24	.23
Average	.93	.94	.90	.94	1.02	.95

## WEEPING WATER.

1	2.82	2.76	2.95	3.07	2.85	2.89
2	1.76	1.74	1.43	2.07	1.75	1.75
3	.80	.73	.59	1.02	.87	.80
4	.47	.48	.45	.56	.44	.48
5	.27	.27	.23	.30	.25	.26
6	.24	.22	.16	.24	.20	.21
Average	1.06	1.03	.97	1.21	1.06	1.06

TABLE XXVIII.

ORGANIC CARBON IN THE FOOT SECTIONS FROM THE DIFFERENT AREAS.

Depth Foot	Wauneta %	McCook %	Holdrege %	Hastings %	Lincoln %	Wpg. Wtr. %	Average %
1	1.61	1.65	2.26	2.06	2.88	2.89	2.22
2	.80	.80	1.08	1.05	1.32	1.75	1.14
3	.63	.56	.60	.57	.66	.80	.64
4	.46	.34	.38	.33	.35	.48	.39
5	.32	.28	.24	.24	.25	.26	.26
6	.26	.21	.21	.18	.23	.21	.22
Average	.68	.64	.79	.74	.95	1.06	.81

TABLE XXIX.

ORGANIC CARBON IN THE DIFFERENT INCH SECTIONS OF THE SURFACE FOOT OF THE SIX DIFFERENT AREAS.

Depth Inch	Wauneta %	McCook %	Holdrege %	Hastings %	Lincoln %	Wpg. Wtr. %	Average %
1	2.85	2.42	4.60	4.52	4.70	4.52	3.93
2	2.11	1.94	3.50	3.17	3.65	3.71	3.01
3	1.85	1.90	2.87	2.58	3.31	3.25	2.63
4	1.67	1.82	2.45	2.28	3.12	3.07	2.40
5	1.48	1.65	2.17	2.03	2.84	2.83	2.17
6	1.46	1.54	2.01	1.86	2.74	2.65	2.04
7	1.31	1.44	1.77	1.79	2.50	2.37	1.86
8	1.27	1.34	1.72	1.67	2.39	2.34	1.79
9	1.23	1.26	1.62	1.59	2.31	2.29	1.72
10	1.14	1.16	1.55	1.53	2.08	2.18	1.61
11	1.03	1.07	1.49	1.52	1.97	2.14	1.54
12	1.03	1.00	1.45	1.45	1.89	2.09	1.48
Average	1.54	1.54	2.25	2.17	2.79	2.79	2.18

TABLE XXX.

AVERAGE CONTENT OF ORGANIC CARBON IN THE FIRST SIX FEET OF EACH FIELD IN EACH OF THE SIX AREAS.

Field No.	Wauneta %	McCook %	Holdrege %	Hastings %	Lincoln %	Wpg. Wtr. %
I	.66	.59	.71	.74	.93	1.06
II	.69	.74	.78	.70	.94	1.03
III	.79	.63	.67	.79	.90	.97
IV	.66	.64	.83	.63	.94	1.21
V	.60	.59	.79	.83	1.02	1.06
Average	.68	.64	.80	.74	.95	1.07

## CARBON-NITROGEN RATIO.

The ratio of the organic carbon to the nitrogen shows some interesting differences. In the surface foot the ratio is everywhere very similar, it varying only between 11.2 and 13.6 for the field samples (Table XXXI). It is independent of the aridity. In the second foot it is lower than in the surface foot, and while it shows a tendency to be lower in the western than in the eastern areas this is not an area characteristic as may be seen from the data for the individual fields. While in most cases the third foot shows a lower ratio than the second, and the fourth one still lower, in neither is an area characteristic exhibited. In the fifth and sixth feet the ratio is still lower, it decreasing less in general in the western areas. As a result of this an accurate analysis of samples from the western, central and eastern areas might be expected to show characteristic differences regularly when the samples analyzed were composites of a very large number of individual samples.

Even in the surface foot the differences in the Carbon-Nitrogen ratio are small (Table XXXIII). It decreases from a maximum varying from 11.9 to 14.3 in the surface inch to a minimum of 10.4 to 11.4 in the twelfth.

## VOLATILE MATTER AND WATER OF CONSTITUTION.

The *volatile matter* as ordinarily determined (6, p. 14) is reported in Table XXXIV together with the organic matter (organic C  $\times$  1.724) and the so-called "water of constitution," which represents the difference between the two preceding values. Instead of the arbitrary 1.724, probably different factors should be used for the different depths; also, judging from the Carbon-Nitrogen ratio, it should not be considered as a constant for even the same depth of subsoil in different fields. However, as nothing more serviceable is as yet available the same factor has been used throughout in calculating the organic matter from the organic carbon. The found percentage of "water of constitution," which in this case represents the water not expelled at 110° C but driven off below a dull red heat, will be affected by any inaccuracy in the determination of organic carbon. As it is derived chiefly from the hydrated silicates and oxides it may be expected to vary as the sum of the alumina and the iron oxide. Considering the average of the six foot depths, it is seen to increase slightly from west to east and in each area to show a maximum in the second or third foot.

The variations are closely concordant with those of the hygroscopicity (Table XXXV). The average ratio of hygroscopic coefficient to water of constitution is 3.43. This ratio varies only between 3.27 and 3.65 for areas, and between 3.40 and 3.53 for the different levels.

TABLE XXXI.  
RATIO OF ORGANIC CARBON TO NITROGEN IN THE FOOT SECTIONS FROM  
THE FIVE FIELDS OF EACH AREA.

## WAUNETA.

Depth Ft.	Field I	Field II	Field III	Field IV	Field V	Average
1	12.4	11.9	11.8	12.0	11.3	11.9
2	10.4	9.6	9.9	9.4	9.3	9.7
3	9.3	11.2	9.3	9.8	8.3	9.6
4	8.9	10.5	12.1	9.6	8.4	9.9
5	8.8	8.0	10.2	6.4	8.4	8.4
6	8.6	8.4	9.4	7.8	8.1	8.5
Average	9.7	9.9	10.5	9.2	9.0	9.7

## McCOOK.

1	11.4	12.4	12.0	12.0	11.3	11.8
2	9.0	10.2	9.7	10.0	8.3	9.4
3	8.8	10.7	10.2	10.6	10.7	10.2
4	8.9	8.2	8.7	8.1	9.1	8.6
5	8.1	9.5	8.0	7.6	10.6	8.8
6	6.9	7.1	7.3	6.6	7.4	7.1
Average	8.8	9.7	9.3	9.1	9.6	9.3

## HOLDREGE.

1	12.2	12.4	13.6	12.3	12.0	12.5
2	10.4	10.7	10.9	10.6	10.7	10.7
3	8.9	8.8	10.1	9.2	9.1	9.2
4	8.7	8.4	8.7	8.4	7.3	8.3
5	6.8	7.6	7.2	6.5	7.7	7.2
6	6.2	6.8	6.7	5.6	4.4	6.0
Average	8.9	9.1	9.5	8.8	8.5	9.0

## HASTINGS.

1	11.8	11.3	11.9	11.4	12.6	11.8
2	11.0	10.6	11.2	10.0	10.8	10.7
3	10.8	10.2	9.0	8.2	11.3	9.9
4	8.4	8.2	8.0	6.6	9.1	8.1
5	7.2	6.9	7.1	5.6	8.3	7.0
6	5.5	5.6	6.1	5.8	7.3	6.1
Average	9.1	8.8	8.9	7.9	9.9	8.9

## LINCOLN.

1	11.9	11.8	11.8	12.1	12.3	12.0
2	10.8	9.1	9.6	10.7	11.0	10.2
3	9.7	8.5	8.5	10.0	10.7	9.5
4	6.7	6.0	5.9	4.8	5.9	5.9
5	4.9	5.3	6.5	5.8	7.5	6.0
6	3.5	5.0	5.2	6.4	6.7	5.4
Average	7.9	7.6	7.9	8.3	9.0	8.2

## WEEPING WATER.

1	12.3	11.9	12.2	12.6	12.0	12.2
2	11.8	11.7	9.8	12.1	11.4	11.4
3	9.9	8.5	7.4	10.5	12.4	9.5
4	8.9	9.1	8.6	7.7	6.9	8.2
5	5.7	6.3	5.6	6.8	6.1	6.1
6	6.0	5.7	4.6	6.4	5.1	5.6
Average	9.1	8.9	8.0	9.3	9.0	8.8

TABLE XXXII.

RATIO OF ORGANIC CARBON TO NITROGEN IN THE DIFFERENT FOOT SECTIONS  
OF THE SIX DIFFERENT AREAS.

Depth Ft.	Wauneta	McCook	Holdrege	Hastings	Lincoln	Wpg.Wtr.	Average
1	11.9	11.8	12.5	11.8	12.0	12.2	12.0
2	9.7	9.4	10.7	10.7	10.2	11.4	10.3
3	9.6	10.2	9.2	9.9	9.5	9.5	9.6
4	9.9	8.6	8.3	8.1	5.9	8.2	8.2
5	8.4	8.8	7.2	7.0	6.0	6.1	7.6
6	8.5	7.1	6.0	6.1	5.4	5.6	6.6
Average	9.7	9.3	9.0	8.9	8.2	8.8	9.1

TABLE XXXIII.

RATIO OF ORGANIC CARBON TO NITROGEN IN THE DIFFERENT FOOT SECTIONS  
OF THE SURFACE FOOT OF THE SIX DIFFERENT AREAS.

Depth In.	Wauneta	McCook	Holdrege	Hastings	Lincoln	Wpg.Wtr.	Average
1	14.3	11.9	14.3	12.8	13.8	13.3	13.4
2	12.2	11.8	13.1	13.4	13.1	12.5	12.8
3	11.2	11.7	12.3	12.6	12.8	12.5	12.3
4	11.2	11.5	11.9	12.3	12.7	12.5	12.0
5	11.0	11.3	11.6	11.6	12.1	12.1	11.6
6	11.4	11.1	11.8	11.5	12.3	11.8	11.6
7	11.1	11.3	11.3	11.5	11.9	10.9	11.3
8	11.3	11.4	11.8	11.1	11.9	11.3	11.5
9	10.9	11.5	11.4	11.0	12.0	11.2	11.3
10	11.0	11.3	11.5	10.9	11.5	11.5	11.3
11	10.5	10.9	11.1	11.1	11.4	11.3	11.0
12	10.6	10.4	11.4	11.0	11.6	11.3	11.0
Average	11.4	11.3	12.0	11.7	12.3	11.9	11.8

## COMPARISON WITH CHERNOZEM SOILS.

The soils of the transition region are, in comparison with the typical Russian Chernozem soils, low in both organic matter and nitrogen. In the surface 4 to 8 inches of the latter, where it has formed upon loess, the organic carbon varies from 3.5 to 6.0 per cent (20, p. 318) and the nitrogen from 0.3 to 0.5 per cent. Both are highest in the central portion of the Chernozem zone and decrease with the approach on one side to the forest regions and on the other to the desert areas. Where the soils have not long been under cultivation these constituents decrease with the depth more or less regularly, although at some distance from the surface there is a rather sharp break, the rate of increase being accelerated.

The two series of analyses from the government of Saratof, given in Table XXVI (20, p. 322) may serve to illustrate the difference in both amount and manner of distribution of the organic matter compared with

TABLE XXXIV.

VOLATILE MATTER, ORGANIC MATTER AND WATER OF CONSTITUTION IN THE FOOT SECTIONS FROM THE DIFFERENT AREAS.

## VOLATILE MATTER.

Depth Foot	Wauneta %	McCook %	Holdrege %	Hastings %	Lincoln %	Wpg.Wtr. %	Average %
1	5.05	5.71	7.10	6.25	8.44	8.43	6.83
2	4.03	4.52	5.10	5.45	6.68	7.17	5.49
3	4.08	3.74	4.48	4.63	5.25	5.24	4.57
4	3.47	3.44	3.98	4.00	4.22	4.46	3.93
5	2.75	3.28	3.34	3.83	4.07	4.27	3.59
6	2.97	3.00	3.10	3.71	3.90	3.99	3.44
Average	3.72	3.97	4.52	4.64	5.43	5.59	4.64

ORGANIC MATTER ( $C \times 1.724$ ).

1	2.77	2.85	3.90	3.55	4.96	4.98	3.83
2	1.38	1.44	1.86	1.81	2.28	3.02	1.96
3	1.09	.97	1.01	.98	1.14	1.38	1.09
4	.79	.59	.66	.60	.60	.81	.68
5	.55	.48	.41	.41	.43	.45	.45
6	.45	.36	.36	.31	.40	.36	.37
Average	1.17	1.11	1.37	1.28	1.63	1.84	1.40

## WATER OF CONSTITUTION.

1	2.28	2.86	3.20	2.70	3.48	3.45	2.99
2	2.65	3.08	3.24	3.64	4.40	4.15	3.53
3	2.99	2.77	3.47	3.65	4.11	3.86	3.48
4	2.68	2.85	3.32	3.40	3.62	3.63	3.24
5	2.20	2.80	2.93	3.42	3.64	3.82	3.14
6	2.52	2.64	2.74	3.40	3.50	3.63	3.07
Average	2.55	2.83	3.15	3.37	3.79	3.76	3.24

TABLE XXXV.

RATIO OF HYGROSCOPIC COEFFICIENT TO WATER OF CONSTITUTION.

Depth Ft.	Wauneta	McCook	Holdrege	Hastings	Lincoln	Wpg.Wtr.	Average
1	3.99	3.50	3.16	3.55	3.45	3.51	3.53
2	3.62	3.54	3.46	3.19	3.27	3.30	3.40
3	3.24	3.77	3.26	3.40	3.31	3.60	3.43
4	3.69	3.40	3.00	3.26	3.59	3.58	3.42
5	4.09	3.25	3.28	3.13	3.52	3.30	3.43
6	3.29	3.45	3.43	3.15	3.63	3.44	3.40
Average	3.65	3.49	3.27	3.28	3.46	3.45	3.43

that in the transition soils. The rate of decrease is similar, but at corresponding depths the amounts are much lower in the latter. The or-

ganic carbon<sup>1</sup> in the first four inches of the Weeping Water and Lincoln areas is barely above the lower limit (3.5 per cent) mentioned above.

The carbon and nitrogen in the Chernozem soils rise and fall together, the ratio being generally somewhat below 11.6 for the surface soil and decreasing from the surface downward (20, p. 323). As this value is based largely upon the analyses of long cultivated soils in which the ratio is perceptibly lower than in virgin soils (3<sub>2</sub>, p. 137; 5, p. 161) it is to be regarded as showing no distinct difference from that in the transition soils.

TABLE XXXVI.

COMPARISON OF THE DISTRIBUTION OF ORGANIC CARBON IN CHERNOZEM SOILS WITH THAT IN THE NEBRASKA LOESS SOILS.

Depth	Chernozem Soils from		Transition Soils from	
	Serdobsk %	Atkarsk %	Weeping Water %	Wauneta %
0-2 inches .....	7.07	....	4.11	2.48
2-4 inches .....	....	6.50	3.16	1.76
4-6 inches .....	6.60	....	2.74	1.47
6-8 inches .....	....	6.50	2.35	1.29
8-10 inches .....	4.54	4.58	2.24	1.18
2nd foot .....	3.56	3.66	1.75	.80
3rd foot .....	2.00	2.08	.80	.63
4th foot .....	1.03	.80	.48	.46

Unfortunately data from systematic fertilizer experiments on the Nebraska loess are not available, but such as there are, those from scattered trials in cooperation with farmers, leave it an open question whether phosphate fertilizers will at present cause any distinct crop increase. However, there appears no doubt that applications of nitrogen fertilizers would increase crop yields, the inadequacy of the supply of available nitrogen in the eastern areas appearing within 20 or 30 years at the most. In this respect the transition soils appear to differ much from the Russian Chernozem soils, which, as mentioned above (p. 202), long retain their fertility, and, when this declines, show a lack first of phosphoric acid, and only later of nitrogen.

<sup>1</sup> The percentages of organic carbon in the Chernozem soils mentioned in this section have been calculated from the "humus" reported by Kossowitsch, who, like all continental Europeans, employs the term as synonymous with our "organic matter of the soil," determined by combustion with copper oxide. In the United States the term *humus* is commonly used to signify only the alkali-soluble portion, although a few Americans use it to signify the whole of the organic matter. Thus Cameron speaks of "the introduction of humus by a grass crop or a green manure crop." (The Soil Solution, 1911, p. 4.)

<sup>2</sup> In Table IV in the reference the carbon in the soil "in cultivation 21 years, in grass 4 years," was, through typographical error reported as 2.10 per cent instead of 3.10.



## COMPARISON WITH ARID SOILS.

It would be of interest to compare the soils of the different areas, and especially those of the two western semi-arid ones, with the arid soils of the United States, but data on the organic carbon and nitrogen content of the latter are rather too scanty to permit of any satisfactory comparison, Hilgard's and Loughridge's extended studies reporting the humus and the humus-nitrogen instead of the organic matter and the total nitrogen.

However, we have data on the relative "rawness" of the subsoils of all the areas. Hilgard has repeatedly called attention to the lack of this in arid subsoils; "cellars and house foundations are dug, and the material removed, even to the depth of 8 feet, is fearlessly put on the garden and there serves as a new soil on which vegetables and small fruits grow, the first year, as well as ever" (18, p. 166). Still more recently (19, p. 418) he writes: "Such a heap now lying before my eyes,<sup>1</sup> the upper layer of which had eight months before been excavated from a depth of 4 meters and still retained the last rain, shows a thick stand of *grasses*<sup>2</sup> and weeds of all kinds, among them wild oats, radish, mustard, . . . ."<sup>3</sup>

We have found, both from pot experiments and by observation in different parts of the loess region where considerable areas of subsoil had been deeply exposed by railroad excavations, that inoculated legumes grow almost as well on the subsoils from depths of 3 to 20 feet as on the corresponding black surface soil, but that non-leguminous plants fail to make any satisfactory growth unless treated with a nitrogen fertilizer or preceded by legume crops. The subsoils of the eastern areas appear no more "raw" than do those of the extreme western. Thus in this respect the semi-arid soils, instead of showing a behavior intermediate between that of the arid soils and that of the humid loess soils, strictly resemble the latter.

## SUMMARY.

The soils studied represent the first six foot-sections, and also the twelve one-inch sections of the surface foot, from five virgin prairie fields in each of six so-called "areas" in Nebraska, located between the Missouri River and the western limit of the loess, a distance of more than 300 miles, in which, while the temperature conditions, wind velocity and relative humidity, are quite uniform, there is a great range in aridity, the annual precipitation decreasing from more than 30 inches in the east to less

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<sup>1</sup> In Berkeley, California.

<sup>2</sup> Italics by the author and not in the original article.

<sup>3</sup> Author's translation from Hilgard, loc. cit.

than 20 in the west, while the relative aridity exhibits a still greater range on account of the increase in the rate of evaporation which accompanies the decrease in precipitation.

The hygroscopicity, as expressed by the hygroscopic coefficient, is strikingly uniform both from field to field in any one area and from the surface downward in the same field. It is lowest in the two western areas and highest in the two eastern. When the different levels from the individual fields are compared, the highest is found in either the second or the third foot, in which two it is very similar. The minimum value is found in the surface foot of the three eastern areas, and in the sixth of the three western. The uniformity within any area is so great that in estimating the free moisture in the first six feet of soil of any field, provided that it be loess, it appears satisfactory to employ simply the average hygroscopic coefficient for all the fields of the area. The effect of the organic matter upon the hygroscopicity is too slight to be detected, a change of even 100 per cent in the content of this being without distinct influence.

The nitrogen content in all the fields decreases from the surface downward. In the surface foot, in which it was determined in each of the twelve inch sections, it decreases steadily, there being in general about half as much in the twelfth as in the first inch section. The nitrogen in the surface foot decreases by about 50 per cent as we pass from the most easterly to the most westerly fields, the difference being such as to permit a definite grouping of the areas. The most easterly areas show as high a content in the second foot as do the most westerly in the first. In this level also there is a decrease from east to west, but it does not show the gradual change exhibited in the first foot. In the still lower levels, third to sixth foot, although the nitrogen in general is higher in the eastern than in the western fields, the differences are small.

The great difference in density of the surface soil from field to field combined with the rapid decrease in nitrogen from the surface downward in virgin prairies, renders satisfactory sampling difficult, which should be carried out in such a way that in sections of like surface equal weights of dry soil are secured.

The organic carbon in the surface foot is very similar in distribution to that of the nitrogen. The amount of the former is approximately 12 times that of the latter, the ratio being uninfluenced by the aridity of the climate. When the inch sections of the surface foot are considered it is seen that the organic carbon decreases slightly more rapidly than does the nitrogen, the average ratio being 13.4 for the first, and 11.3 for the twelfth inch section. In the levels below the first foot also a similar difference in the rate of decrease is observed, the ratio in some cases falling as low as 6.0. The decrease is less rapid in the western than in the east-

ern areas, the average organic carbon content in the fourth, fifth, and sixth feet being higher in the two most westerly areas than in the two most easterly, while that of the nitrogen is lower.

The decrease in nitrogen and organic carbon in the surface soil as we proceed from east to west cannot be explained by the increase in coarseness of texture, but must be attributed to the greater vegetative growth without a correspondingly more rapid decay in the eastern areas.

The water of constitution—the difference between volatile matter and organic matter—decreases from east to west, the variations being concordant with those in the hygroscopicity.

Compared with the Russian Chernozem soils formed on loess the organic carbon and the nitrogen are low both in the surface soil and in the subsoil, the amounts found in the eastern areas being similar to the minima reported for the Chernozem.

The subsoils from the semi-arid areas, in so far as the nitrogen is concerned, in contrast with the arid subsoils, are as "raw" as those from the humid areas, not supporting a satisfactory growth of non-leguminous plants.

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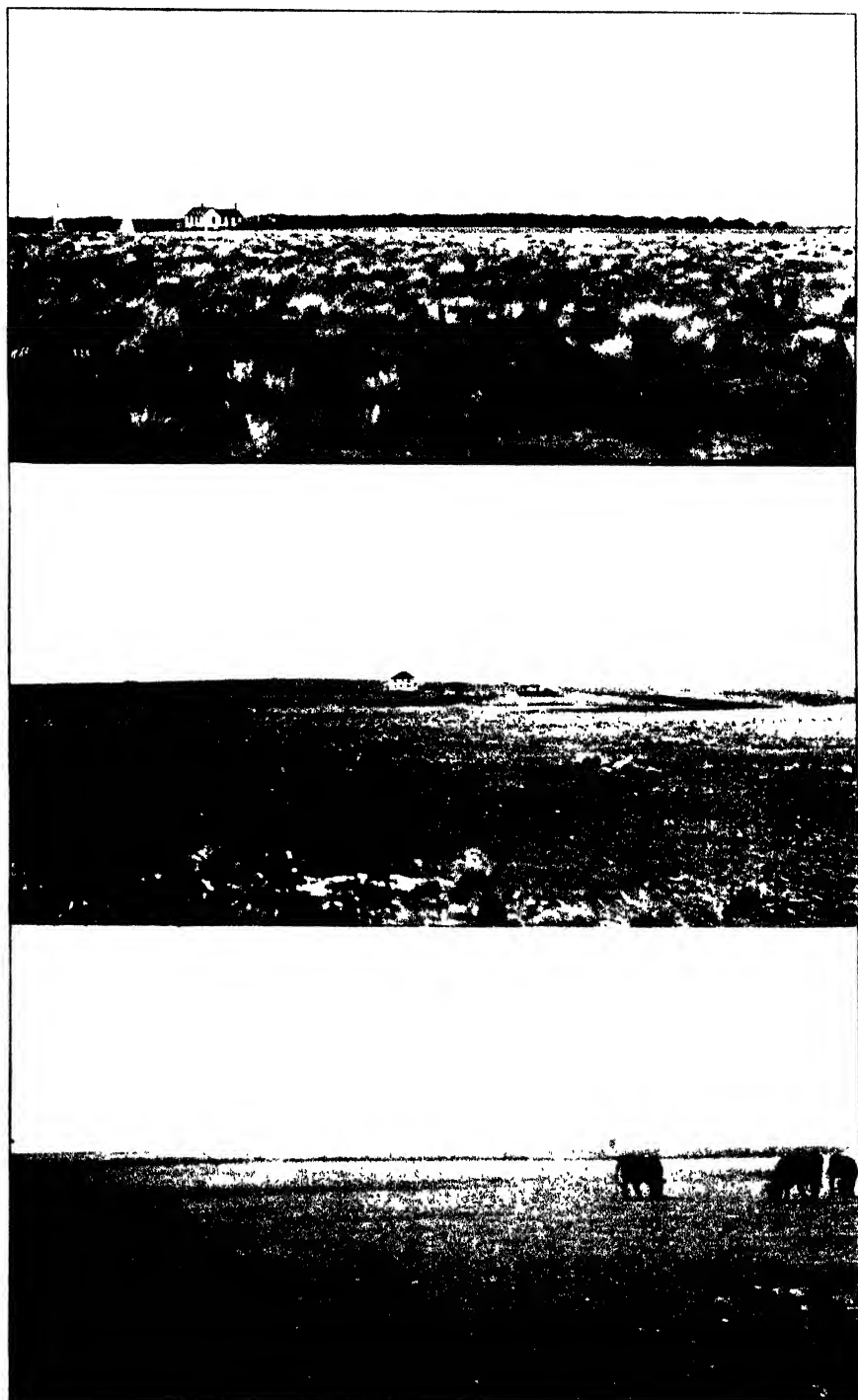


PLATE I.

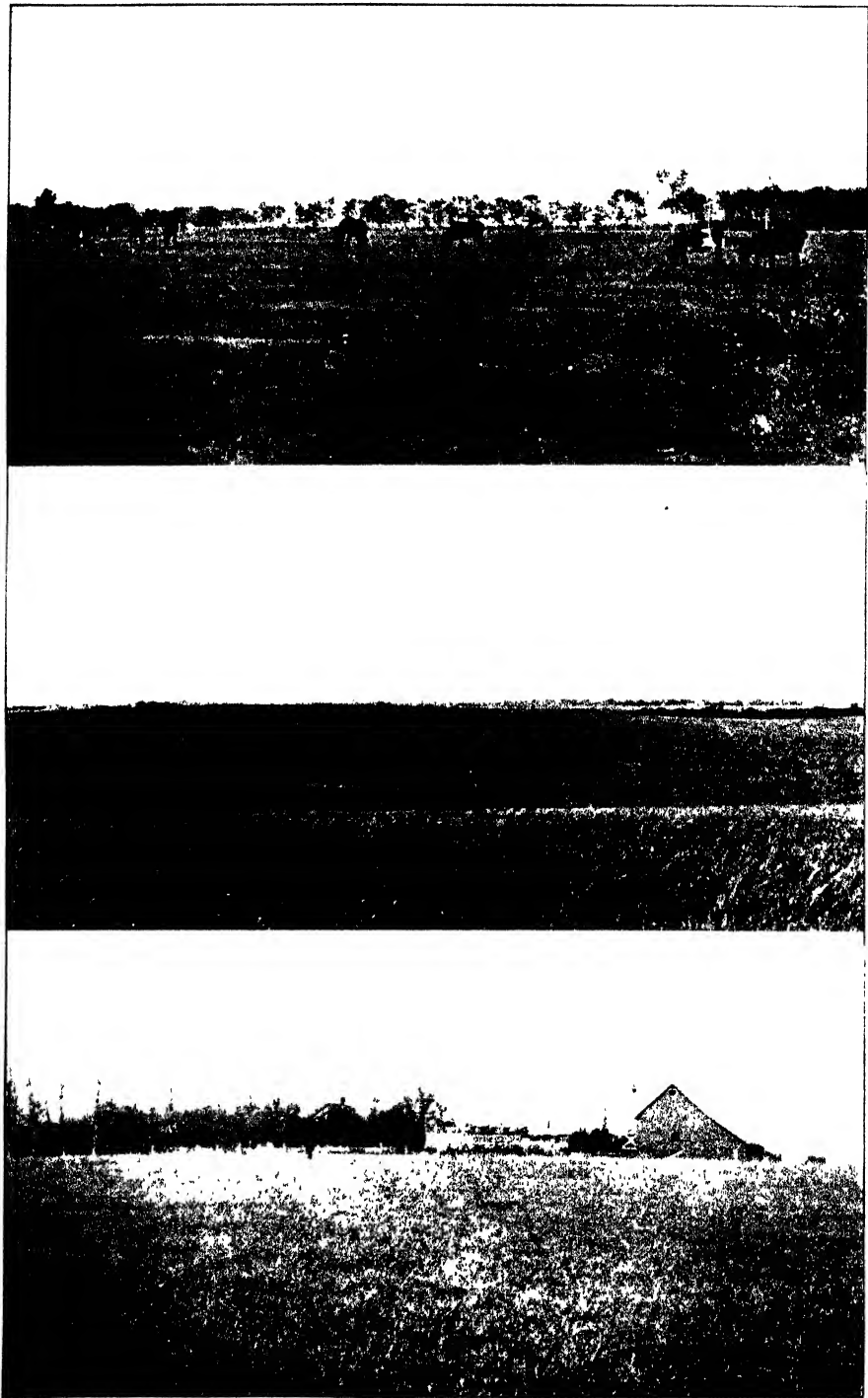
Fig. 1.—Field II at Wauneta showing very level character of fields in this area.  
A young orchard is shown in the foreground.

Fig. 2.—Field III at McCook. Planted trees about the farmstead. Canyons in the  
loess at the right.

Fig. 3.—Field V at Holdredge, showing level topography. Planted trees in the  
distance.







**PLATE II.**

**Fig. 1.—Field IV at Hastings, showing very level topography. Planted trees in the distance.**

**Fig. 2.—Field IV at Lincoln, showing rolling topography and, in the distance, native trees along water-courses.**

**Fig. 3.—Field V at Weeping Water, showing orchard and shade trees about a farmstead. This was one of the very few comparatively level tracts that had not been brought under the plow.**

### PLATE III.

- Fig. 1.**—At the western edge of the loess plain. Looking westward from Field IV at Wauneta, showing the lower-lying plain of Tertiary rocks covered with residual soil. The loess extends about 300 yards beyond the immediate foreground.
- Fig. 2.**—A canyon in the loess between Fields II and IV at Wauneta, showing contact of loess with underlying unaltered Tertiary rock. The man is shown standing on a slight projection of the latter. The photograph was taken from the opposite side of the canyon.





# THE LOESS SOILS OF THE NEBRASKA PORTION OF THE TRANSITION REGION :

## II. HUMUS, HUMUS-NITROGEN AND COLOR.<sup>1</sup>

By

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### INTRODUCTION.

In Nebraska the loess extends westward from the Missouri River for about 300 miles. Throughout this distance the temperature conditions are quite uniform, but there is a gradual decrease in the humidity of the climate, the normal annual precipitation, which exceeds 30 inches at the eastern boundary, steadily falling until it is less than 20 in the extreme western portion, while the rate of evaporation increases considerably. The climate of this region has been considered in detail in a previous paper (3).

The soil samples upon which the present article is based, were collected from 30 virgin prairie fields, 5 near each of six stations of the United States Weather Bureau shown in figure 1—Wauneta, McCook, Holdrege, Hastings, Lincoln and Weeping Water. In each field, at intervals of 30 feet, ten borings were made to a depth of six feet and composite samples prepared of each foot section, thus giving six samples from each field, the so-called "field samples." From these "area samples" were prepared by mixing equal weights of the corresponding 5 "field foot samples." Thus each of the "area samples" is a composite from 50 individual borings. In addition to this a set of 12 one inch samples was secured from the surface foot of each field, these being composites of 20 or 50 individual samples. The "area inch samples" are accordingly composites of 100 or 250 individual samples. The details of the method of sampling are given in the article above referred to.

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<sup>1</sup> Received for publication February 10, 1916.

<sup>2</sup> The work reported in this paper was carried out at the Nebraska Agricultural Experiment Station, where the authors were Chemist and Assistant in Chemistry, respectively.

## HUMUS.

"Humus" as used in this article refers to the *matière noire* of Grandeu (7, p. 148)—the portion of the soil soluble in 4 per cent ammonium hydroxide solution after previous treatment with 1 per cent hydrochloric acid. We have studied the distribution of this in the different foot levels, and by inch sections in the surface foot. In the first foot samples from all the fields we have determined the humus-nitrogen to see whether the proportion is distinctly higher in the semi-arid than in the humid soils. The constancy of the ratio of humus to the total nitrogen and to the organic carbon, and also the relation of the humus, the organic carbon and the total nitrogen to the soil color have been investigated.

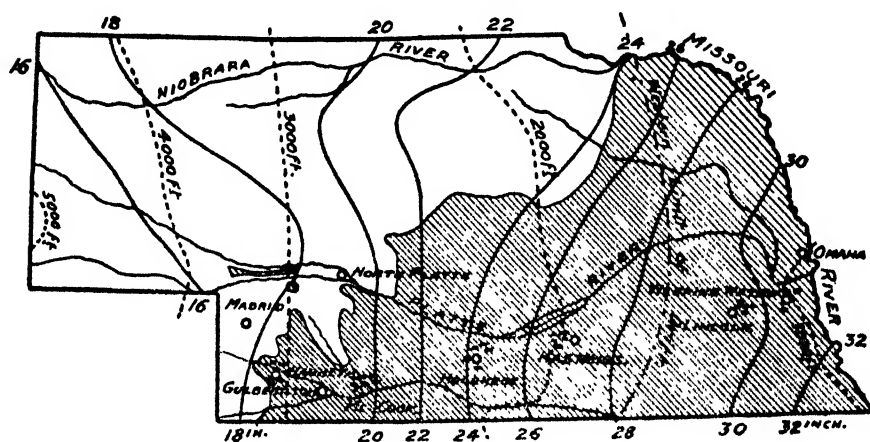


Figure 1—Map of Nebraska showing distribution of the loess (shaded), annual precipitation and location of fields sampled.

It is now generally recognized that a large number of the past determinations of humus, as this term is above defined, are unreliable because of the failure to recognize the influence of the relative amounts of the so-called "humus-ash" upon the accuracy of the determination (2, p. 322; 6, p. 56). As a high percentage of ash indicates an unreliable determination we report the amount of this wherever we employ data from gravimetric determinations.

Several reliable methods for the determination of humus are available. We compared that of Rather (12) with Hilgard (8, p. 246), and Moores-Hampton (11) methods and since it gave similar results, while requiring less time, adopted it in this study. In the case of the surface soils from the same locality it has been found that the intensity of the color of the ammonia extract is fairly closely concordant with the amount of humus contained (4). This permits of the use of a colori-

TABLE I.  
HUMUS IN THE FOOT SECTIONS FROM THE FIVE FIELDS OF EACH AREA,  
DETERMINED COLORIMETRICALLY.

## WAUNETA.

Depth Foot	Field I %	Field II %	Field III %	Field IV %	Field V %	Average %
1	1.17	1.00	1.17	1.00	1.00	1.07
2	.87	.78	.87	.93	.74	.84
3	.74	1.08	.74	.93	.50	.80
4	.39	1.00	.70	.58	.34	.60
5	.26	.31	.87	.26	.20	.38
6	.18	.19	.82	.15	.17	.30
Average	.60	.73	.86	.64	.49	.66

## McCOOK.

1	.93	1.48	1.17	1.08	1.00	1.13
2	.52	1.08	.61	.82	.40	.69
3	.39	1.04	.33	.45	.19	.48
4	.33	.82	.22	.24	.18	.36
5	.19	.30	.31	.19	.17	.23
6	.21	.19	.24	.16	.14	.19
Average	.43	.82	.48	.49	.35	.51

## HOLDREGE.

1	1.48	1.65	1.75	2.00	2.15	1.81
2	.78	1.04	1.35	1.17	1.17	1.10
3	.37	.44	.66	.70	.39	.51
4	.22	.25	.44	.58	.24	.34
5	.16	.19	.22	.16	.15	.18
6	.13	.15	.20	.10	.13	.14
Average	.51	.62	.77	.78	.71	.68

## HASTINGS.

1	1.40	1.67	1.26	1.55	1.55	1.49
2	.82	.92	.82	1.00	.82	.88
3	.42	.50	.40	.36	.28	.39
4	.26	.41	.17	.30	.15	.26
5	.23	.41	.14	.29	.13	.24
6	.13	.30	.10	.15	.12	.16
Average	.54	.70	.48	.61	.51	.56

## LINCOLN.

1	2.55	2.55	2.15	2.33	2.33	2.38
2	.87	.64	.30	.56	.84	.64
3	.17	.13	.11	.23	.26	.18
4	.09	.08	.06	.12	.12	.09
5	.04	.05	.05	.09	.10	.07
6	.05	.07	.05	.06	.09	.06
Average	.63	.59	.45	.56	.62	.57

## WEEPING WATER.

1	2.15	2.00	2.33	2.55	2.15	2.24
2	.70	.72	.72	1.40	.78	.86
3	.14	.10	.15	.14	.11	.13
4	.07	.08	.11	.10	.09	.09
5	.06	.06	.04	.08	.08	.06
6	.06	.05	.04	.04	.04	.05
Average	.53	.50	.56	.72	.54	.57



metric method, which is far more expeditious. In comparing humid and semi-arid subsoils we have found that the former may give an almost colorless and the latter a brown ammonia extract, while the amount of humus determined by the gravimetric method is practically the same in both. Even when the ammonia extract is almost colorless a gravimetric determination may show from 0.15 to 0.20 per cent humus with a humus ash equal to that in soils of high humus content. As a satisfactory method should indicate at least the relative amounts of the dissolved black substances we consider that the colorimetric method is altogether preferable for the subsoils, although the two methods appear about equally satisfactory for the surface soils.

TABLE II.

HUMUS IN THE FIRST AND SECOND FOOT SECTIONS FROM THE FIVE FIELDS OF EACH AREA, DETERMINED GRAVIMETRICALLY.

## WAUNETA.

Depth Foot	Field I %	Field II %	Field III %	Field IV %	Field V %	Average %
1	0.99	1.04	1.07	0.99	1.02	1.02
2	.65	.61	.72	.67	.64	.66

## McCOOK.

1	1.12	1.27	1.15	1.15	1.04	1.15
2	.55	.81	.60	.67	.49	.62

## HOLDREGE.

1	1.37	1.44	1.63	1.70	1.90	1.61
2	.69	.79	1.01	.95	.93	.87

## HASTINGS.

1	1.50	1.67	1.39	1.56	1.42	1.51
2	.85	.92	.84	.91	.79	.86

## LINCOLN.

1	2.30	2.22	2.19	2.27	2.34	2.26
2	1.08	.96	.80	.90	1.16	.98

## WEEPING WATER.

1	2.13	2.09	2.24	2.43	2.28	2.23
2	1.18	1.30	1.08	1.65	1.27	1.29

While part of the samples have been subjected to only the gravimetric and part to only the colorimetric, both methods were used with the majority. As the surface soils, in general, give similar results by both we used only the gravimetric for the inch sections. Both methods were used with all the area foot samples and with the field samples from the first and second foot. The colorimetric method only was employed with

TABLE III.  
"HUMUS ASH" FROM THE FIRST AND SECOND FOOT OF THE FIVE FIELDS OF  
EACH AREA.

## WAUNETA.

Depth Foot	Field I %	Field II %	Field III %	Field IV %	Field V %	Average %
1	.40	.33	.45	.34	.42	.39
2	.34	.27	.35	.32	.32	.32

## McCOOK.

1	.29	.32	.30	.29	.26	.29
2	.35	.27	.36	.31	.36	.33

## HOLDREGE.

1	.30	.29	.29	.34	.29	.30
2	.25	.23	.30	.28	.28	.27

## HASTINGS.

1	.24	.19	.20	.27	.23	.23
2	.27	.22	.27	.21	.22	.24

## LINCOLN.

1	.26	.38	.25	.22	.31	.28
2	.43	.18	.15	.19	.16	.22

## WEEPING WATER.

1	.29	.28	.36	.32	.37	.32
2	.16	.16	.14	.34	.18	.20

TABLE IV.  
HUMUS IN THE FOOT SECTIONS FROM THE DIFFERENT AREAS.

## COLORIMETRIC DETERMINATION.

Depth Foot	Wauneta %	McCook %	Holdrege %	Hastings %	Lincoln %	Wpg.Wtr. %	Average %
1	1.07	1.13	1.81	1.49	2.38	2.24	1.69
2	.84	.69	1.10	.88	.64	.86	.83
3	.80	.48	.51	.39	.18	.13	.42
4	.60	.36	.34	.23	.09	.09	.29
5	.38	.23	.18	.22	.07	.06	.19
6	.30	.19	.14	.14	.06	.05	.15
Average	.66	.51	.68	.56	.57	.57	.59

## GRAVIMETRIC DETERMINATION.

1	1.02	1.15	1.61	1.51	2.26	2.24	1.63
2	.65	.62	.87	.86	.98	1.29	.88
3	.48	.35	.33	.35	.38	.55	.41
4	.34	.31	.29	.26	.26	.27	.29
5	.26	.27	.21	.28	.21	.23	.24
6	.26	.27	.18	.25	.15	.19	.22
Average	.50	.49	.58	.58	.71	.80	.61

the field foot samples from the lower levels. Thus we have data upon which to base a fair comparison of the two methods. We confirmed the earlier observations that with the colorimetric method better results are obtained when the standard used is a soil of the same type and from the same locality as the soils under investigation (4, p. 14).

TABLE V.  
"HUMUS ASH" FROM THE AREA COMPOSITES.

Depth Foot	Wauneta %	McCook %	Holdrege %	Hastings %	Lincoln %	Wpg.Wtr. %
1	.39	.29	.30	.23	.28	.32
2	.32	.33	.27	.24	.22	.20
3	.37	.46	.34	.27	.19	.23
4	.45	.52	.37	.40	.25	.19
5	.53	.54	.37	.40	.24	.27
6	.60	.54	.39	.44	.34	.27

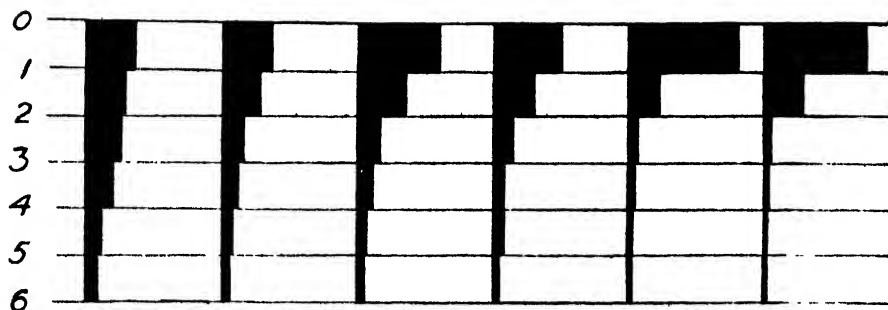
In Table I there is reported the humus content of the foot samples from the five fields in each of the six areas, as determined by the colorimetric method. As a standard we used the surface six inches of soil from one of the long cultivated fields on the experiment station farm. This contained 0.244 per cent nitrogen, 2.20 per cent humus and had a hygroscopic coefficient of 10.2.

In the case of all the field samples from the first and second foot the humus was determined by the gravimetric method also (Tables II and III). The same method was applied to the area composites of the lower four feet. In Tables IV and V the data for the area samples as obtained by the two methods are reported. Except in the case of the gravimetric determinations for depths below the second foot they are the averages for the field samples. Figure 2 shows graphically the distribution of humus as determined by the two methods. For the first foot samples the two methods give quite similar results and, as with the total nitrogen and the organic carbon (3, p. 226), the fields may be arranged in three groups, that highest in humus including those from Weeping Water and Lincoln, and that lowest those near Wauneta and McCook. For the second foot samples no such grouping is possible, and in the case of these the colorimetric method shows lower percentages than the gravimetric for all Weeping Water and Lincoln fields, while for those of the western four areas first the one method and then the other gives the higher results. For the levels below the second foot the colorimetric determinations show an increase in humus from east to west while the gravimetric show a quite uniform distribution. Considering the averages for the six feet the colorimetric determinations show no change from east to west, while the gravimetric indicate a distinct decrease.

Where, as in the present study, part of the determinations of humus have been made by the gravimetric method, and so represent the whole of the ammonia-soluble organic matter, and part by the colorimetric method and thus indicate merely the relative amounts of pigment, it is necessary, in order to avoid confusion, to indicate, wherever the term *humus* is employed, which method has been used. It might be preferable to continue to use this term to indicate simply the ammonia-soluble organic matter without regard to its color and to refer to values obtained by the colorimetric method as *soluble pigment*. From the discussion in the following paragraphs it will be seen that this probably constitutes an indefinite portion of the organic matter and at most carries but a small portion of the soil nitrogen. Hence the percentages of soluble pigment as reported in the tables are to be regarded as indicating not the absolute, but only the relative, amounts present. Until the pigment has been isolated it will not be possible to secure a standard solution by means of which the actual percentages can be determined.

*Ft. Wauneta McCook Holdrege Hastings Lincoln W. Water.*

### COLORIMETRIC.



### GRAVIMETRIC.

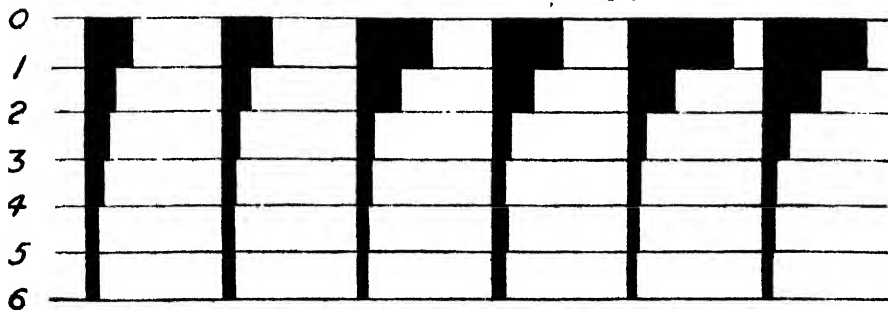


Figure 2—Diagram showing relative amounts of humus found by the two methods in the different areas.

When we employ the data from the colorimetric method we can sharply distinguish the fields of the most westerly, semi-arid two areas from the most easterly, humid two. The former are characterized by a content of soluble pigment lower in the surface foot but much higher in the third and lower foot sections (fig. 3).

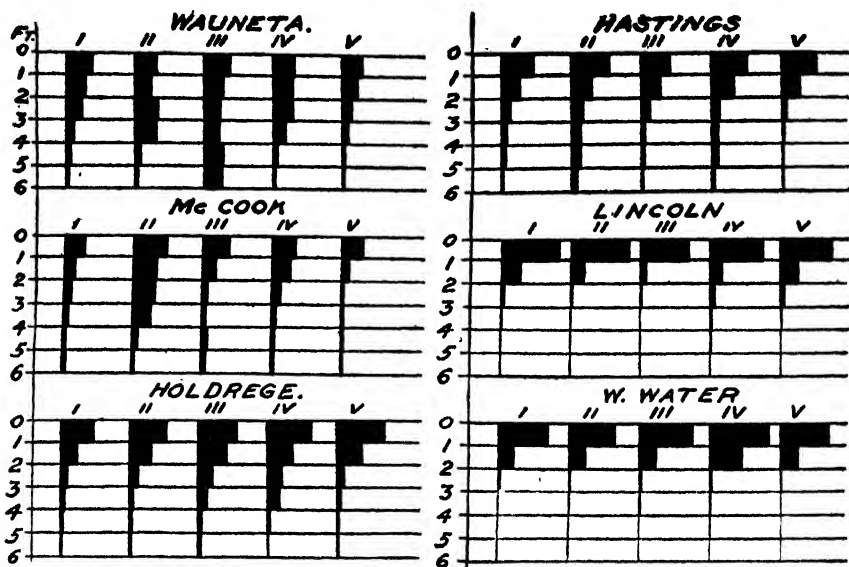


Figure 3—Diagram showing the distribution of humus, as determined colorimetrically, in the different fields.

In the western four areas, and at McCook and Wauneta especially, some of the fields show an exceptionally high content of soluble pigment at levels below the second foot. In Field II at McCook and II at Wauneta this continues through the fourth foot, and in III at Wauneta through the sixth. There is nothing found in the topography, drainage, etc. of these fields that offers any explanation of their exceptional composition. At the time the samples were taken we noticed the darker color of the subsoil, and later Field II at McCook was again visited and carefully examined to make sure that the samples were in color strictly representative of the lower levels. This exceptionally high content of soluble pigment in the deeper subsoil, 3 to 6 feet, exhibited by the three fields mentioned, while not an area characteristic, has been found only in the western areas. As all four western areas show a higher content at these depths than the eastern two it seems not improbable that whatever has caused the high content of soluble pigment in all the western fields has also been the cause of the exceptional amounts found in the three above mentioned.

From the data on the total nitrogen (3, p. 220), it will be seen that these exceptional amounts of soluble pigment are not accompanied by correspondingly high percentages of nitrogen, although the latter are above the average.

The humus in the area inch samples, determined gravimetrically, is reported in Table VI. It decreases from east to west and in all the areas decreases quite uniformly from the surface downward, the relative

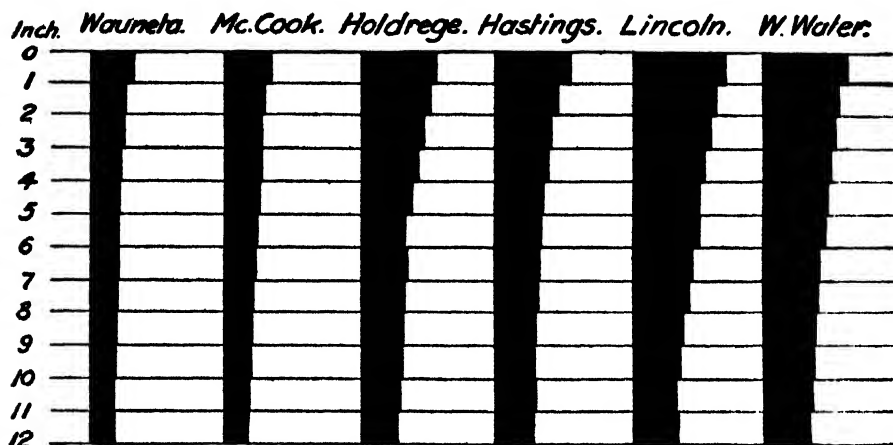


Figure 4—Diagram showing the distribution of humus in the surface foot.

change, inch by inch, being quite similar in all (fig. 4). The "humus ash" (Table VII) is highest in the surface inch, which may simply indicate that the ammonia solution dissolved more mineral matter from this section. If this ash were derived from phosphorus, potassium, etc., forming an essential part of definite organic compounds, it should be highest in the first inch samples from the eastern two areas, which is not the case. In these it amounts to less than one-fifth the humus, but in the McCook and Wauneta samples to more than one-half.

#### THE RATIO OF HUMUS TO NITROGEN.

The total nitrogen and the organic carbon in all these samples has been previously reported (3, p. 226). The ratio of humus to nitrogen in the first and the second foot of all the fields, using the data from the gravimetric determinations, is reported in Table VIII. In the case of the surface foot the ratio averages slightly the highest in the eastern two areas, but for some of the fields in these it is lower than for certain fields in the intermediate two. All the fields in the western two areas show a lower ratio than those of the eastern two. The average for all the fields

for the second foot, 8.1, is similar to that for the first foot, 8.7. In all the fields of the eastern two areas the ratio is lower in the second than in the first foot, while in those of the other areas it is sometimes higher and sometimes lower.

TABLE VI.

HUMUS IN THE INCH SECTIONS OF THE SURFACE FOOT, DETERMINED GRAVIMETRICALLY.

Depth Inch	Wauneta %	McCook %	Holdrege %	Hastings %	Lincoln %	Wpg.Wtr. %	Average %
1	1.42	1.50	2.46	2.57	3.06	2.85	2.31
2	1.16	1.26	2.24	2.11	2.75	2.54	2.01
3	1.11	1.20	2.06	1.84	2.59	2.42	1.87
4	1.02	1.20	1.89	1.75	2.36	2.28	1.75
5	.98	1.12	1.65	1.58	2.17	2.17	1.61
6	.97	1.06	1.42	1.49	2.17	2.12	1.54
7	.97	1.01	1.50	1.48	1.97	1.94	1.48
8	.90	.94	1.38	1.44	1.89	1.88	1.41
9	.87	.87	1.36	1.33	1.65	1.79	1.31
10	.81	.85	1.32	1.30	1.58	1.77	1.27
11	.78	.79	1.27	1.33	1.45	1.68	1.22
12	.80	.76	1.19	1.25	1.47	1.56	1.17
Average	.98	1.05	1.65	1.62	2.10	2.10	1.58

TABLE VII.

"HUMUS ASH" FROM THE DIFFERENT INCH SECTIONS OF THE SURFACE FOOT.

Depth Inch	Wauneta %	McCook %	Holdrege %	Hastings %	Lincoln %	Wpg.Wtr. %	Average %
1	.96	.84	.77	.73	.47	.52	.71
2	.55	.43	.59	.50	.40	.40	.48
3	.42	.35	.35	.32	.40	.39	.37
4	.32	.32	.31	.36	.31	.29	.32
5	.31	.31	.30	.29	.35	.31	.31
6	.31	.30	.31	.23	.35	.34	.31
7	.31	.29	.30	.22	.33	.26	.29
8	.30	.29	.31	.27	.35	.27	.30
9	.33	.29	.30	.22	.27	.25	.28
10	.35	.29	.29	.29	.27	.28	.29
11	.31	.29	.30	.25	.26	.23	.27
12	.31	.29	.29	.22	.30	.24	.28
Average	.40	.36	.37	.33	.34	.32	.35

The substitution of the data from the colorimetric determination (Table IX) affects the ratios very much less in the case of the first two feet than in that of the lower sections. For the latter the resulting ratios at Lincoln and Weeping Water are in most cases less than 2, while those for the more westerly areas are in general far above this, values of 12 or even higher being found. Thus it is evident that the amount of the dark colored, ammonia-soluble matter shows no definite relation to the total

nitrogen content, a condition which would indicate that the substance or substances causing the black or brown color of the ammonia extract contain at most but a very small proportion of the total nitrogen of the soil.

In the inch sections of the surface foot the ratio of humus, as determined gravimetrically, to nitrogen (Table X), is somewhat lower in the western two areas, averaging between 7 and 8, while in the other four the average is approximately 9.

TABLE VIII.

RATIO OF HUMUS, AS DETERMINED GRAVIMETRICALLY, TO NITROGEN IN THE FIRST TWO FEET OF EACH OF THE FIELDS.

FIRST FOOT.						
Field No.	Wauneta	McCook	Holdrege	Hastings	Lincoln	Wpg.Wtr.
I	7.3	7.8	8.0	8.8	9.5	9.3
II	7.5	8.7	8.3	9.6	9.1	9.0
III	7.4	8.3	9.9	7.6	9.4	9.2
IV	7.7	8.0	9.0	9.2	9.5	10.0
V	7.7	8.3	9.1	8.2	9.7	9.2
Average	7.5	8.3	8.9	8.6	9.4	9.5
SECOND FOOT.						
I	8.1	7.0	7.7	8.9	8.8	7.9
II	7.8	9.0	8.1	9.7	6.6	8.7
III	7.8	7.5	9.1	8.2	6.4	7.4
IV	8.2	7.6	9.1	9.8	7.6	9.6
V	8.1	5.8	9.0	7.6	8.7	8.3
Average	7.9	7.4	8.6	8.8	7.6	8.4

#### ORGANIC CARBON-HUMUS RATIO.

The ratio of organic carbon to humus in the inch sections of the surface foot is shown in Table XI. It averages slightly the highest in the western areas. In each of the six areas it is highest in the surface inch. In the lowest sections it is alike in the most westerly and the most easterly areas. The average for all the sections in all the areas is 1.4. Accordingly the ratio of organic matter ( $C \times 1.7241$ ) to humus will be 1:2.4; in other words about 40 per cent of the organic matter of the surface foot is soluble in a 4 per cent ammonia solution.

In the area foot samples (Table XII) the ratio of organic carbon to humus, as determined by the gravimetric method, is fairly constant in the first two feet, rising slightly from east to west in the first, as already shown in the series of inch sections. For the lower foot sections while the average is the same as for the first two feet the ratio varies from 0.7 to 1.8. Using the colorimetric determinations the ratio in the third, fourth, fifth and sixth foot varies from *ca.* 1.0 to 1.5 in the western four areas, and from *ca.* 3.5 to 6.0 in eastern two (Table XII).



TABLE IX.  
RATIO OF HUMUS, DETERMINED COLORIMETRICALLY, TO NITROGEN.

WAUNETA.

Depth Ft.	Field I	Field II	Field III	Field IV	Field V	Average
1	8.1	7.3	8.1	7.7	7.6	7.8
2	10.9	10.0	9.5	11.3	9.4	10.2
3	12.5	18.6	10.3	11.9	8.3	12.3
4	9.1	17.9	16.7	12.3	7.9	12.8
5	7.9	8.9	17.8	6.7	5.6	9.4
6	6.4	7.6	16.7	6.5	6.3	8.7

McCOOK.

1	6.5	10.1	8.5	7.5	8.0	8.1
2	6.6	12.0	7.6	9.3	4.4	8.0
3	9.1	15.5	6.3	8.6	3.9	8.7
4	9.2	16.7	5.9	6.7	5.3	8.8
5	6.1	8.1	9.1	6.5	5.4	7.0
6	6.2	6.3	8.0	5.5	5.2	6.2

HOLDREGE.

1	8.6	9.5	10.4	10.6	10.3	9.9
2	8.8	10.6	12.0	11.2	11.3	10.8
3	6.7	6.9	8.9	9.3	7.1	8.0
4	6.1	5.8	8.3	10.4	6.5	7.2
5	5.2	5.0	5.5	5.0	5.6	5.3
6	3.8	4.4	5.1	2.9	4.6	4.2

HASTINGS.

1	8.2	9.6	6.9	9.2	8.9	8.6
2	8.6	9.7	8.0	10.8	7.9	9.0
3	7.1	9.4	6.4	6.7	4.7	6.9
4	6.0	10.5	3.9	9.4	3.3	6.6
5	5.3	14.1	3.4	11.6	3.2	7.5
6	4.5	11.1	3.0	6.2	3.5	5.7

LINCOLN.

1	10.6	10.4	9.2	9.8	9.6	9.9
2	7.1	4.4	2.4	4.6	6.3	5.0
3	2.5	1.8	1.5	3.6	3.6	2.6
4	1.8	1.3	1.0	1.9	1.8	1.6
5	.9	1.1	1.2	2.2	2.8	1.6
6	.9	1.5	1.2	1.5	2.5	1.5

WEeping WATER.

1	9.4	8.7	9.6	10.5	9.1	9.5
2	4.7	4.8	4.9	8.2	5.1	5.6
3	1.7	1.2	1.9	1.4	1.6	1.6
4	1.3	1.5	2.1	1.4	1.4	1.5
5	1.3	1.4	1.0	1.8	1.9	1.5
6	1.5	1.3	1.1	1.0	1.0	1.2

TABLE X.

RATIO OF HUMUS, AS DETERMINED GRAVIMETRICALLY, TO NITROGEN IN THE INCH SECTIONS OF THE SURFACE FOOT.

Depth In.	Wauneta	McCook	Holdrege	Hastings	Lincoln	Wpg.Wtr.	Average
1	7.1	7.4	7.6	7.3	9.0	8.4	7.8
2	6.7	7.6	8.4	8.9	9.5	8.6	8.3
3	6.7	7.3	8.8	8.9	10.0	9.3	8.5
4	6.8	7.5	9.2	9.4	9.6	8.3	8.5
5	7.3	7.7	8.8	9.0	9.3	9.3	8.6
6	7.6	7.6	8.4	9.2	9.7	9.4	8.6
7	8.2	7.9	9.6	9.5	9.4	8.9	8.9
8	8.0	8.0	9.5	9.6	9.4	9.0	8.9
9	7.7	7.9	9.6	9.2	8.5	8.7	8.6
10	7.8	8.3	9.7	9.3	8.7	8.9	8.8
11	8.0	8.2	9.5	9.7	8.4	8.8	8.8
12	8.4	8.3	9.4	9.5	9.0	8.4	8.8
Average	7.5	7.8	9.0	9.1	9.2	9.1	8.7

TABLE XI.

RATIO OF ORGANIC CARBON TO HUMUS, AS DETERMINED GRAVIMETRICALLY, IN THE DIFFERENT INCH SECTIONS OF THE SURFACE FOOT OF THE SIX DIFFERENT AREAS.

Depth In.	Wauneta	McCook	Holdrege	Hastings	Lincoln	Wpg.Wtr.	Average
1	2.0	1.6	1.9	1.8	1.5	1.6	1.7
2	1.8	1.5	1.6	1.5	1.3	1.5	1.5
3	1.7	1.6	1.4	1.4	1.3	1.3	1.4
4	1.6	1.5	1.3	1.3	1.3	1.3	1.4
5	1.5	1.5	1.3	1.3	1.3	1.3	1.4
6	1.5	1.5	1.4	1.2	1.3	1.3	1.3
7	1.4	1.4	1.2	1.2	1.3	1.2	1.4
8	1.4	1.4	1.2	1.2	1.3	1.2	1.3
9	1.4	1.5	1.2	1.2	1.4	1.3	1.3
10	1.4	1.4	1.2	1.2	1.3	1.3	1.3
11	1.3	1.4	1.2	1.1	1.4	1.3	1.3
12	1.3	1.3	1.2	1.2	1.3	1.3	1.3
Average	1.5	1.5	1.3	1.3	1.3	1.3	1.4

As the total organic matter in the lower levels is quite similar in all the areas, as is also the ammonia soluble portion—the humus as determined gravimetrically—the change from east to west is apparently confined chiefly to the amount of pigment, which forms a quite unknown proportion of the dissolved organic matter.

#### PERCENTAGE OF NITROGEN IN THE HUMUS.

For the extraction of the humus-nitrogen 10 gm. of dry soil was placed in a glass-stoppered flask and treated with 500 c.c. 4 per cent potassium hydroxide solution. The mixture was shaken at frequent intervals for 8 days, after which it was allowed to stand over night that the suspended soil particles might settle. An aliquot of this dark colored ex-

tract was used for the Kjeldahl determination. This method (1) is much more convenient and expeditious than that of Hilgard (8, p. 247), while extracting as large a proportion of the soil nitrogen.

TABLE XII.  
RATIO OF ORGANIC CARBON TO HUMUS IN THE FOOT SECTIONS.

A.—HUMUS DETERMINED BY COLORIMETRIC METHOD.

Depth Ft.	Wauneta	McCook	Holdrege	Hastings	Lincoln	Wpg.Wtr.	Average
1	1.5	1.5	1.2	1.4	1.2	1.3	1.3
2	.9	1.2	1.0	1.2	2.7	2.0	1.5
3	.8	1.2	1.2	1.5	3.7	6.1	2.4
4	.8	.9	1.1	1.5	3.9	5.3	2.2
5	.8	1.2	1.3	1.1	3.6	4.3	2.1
6	.9	1.1	1.5	1.3	3.8	4.2	2.1

B.—HUMUS DETERMINED BY GRAVIMETRIC METHOD.

1	1.6	1.6	1.4	1.4	1.3	1.3	1.4
2	1.2	1.3	1.2	1.2	1.3	1.4	1.3
3	1.3	1.6	1.8	1.6	1.7	1.4	1.6
4	1.4	1.1	1.3	1.3	1.3	1.8	1.4
5	1.2	1.1	1.1	.9	1.2	1.1	1.1
6	1.0	.8	1.2	.7	1.5	1.1	1.0

The percentage of nitrogen in the humus, based upon the gravimetric determinations of the latter, in the surface foot of each field, is reported in Table XIII. It averages lower for the eastern two than for the other areas, but the differences are so small, being no greater than the variations within the different areas, that the data justify little hope of finding in the semi-arid region soils with humus showing an abnormally high content of nitrogen. Recent work has rendered it probable that even the arid soils as a rule are not characterized by a high content of nitrogen in the humus (1).

TABLE XIII.  
PERCENTAGE OF NITROGEN IN THE HUMUS FROM THE FIRST FOOT OF THE DIFFERENT FIELDS.

Field No.	Wauneta %	McCook %	Holdrege %	Hastings %	Lincoln %	Wpg.Wtr. %
I	8.3	8.0	7.6	8.4	6.6	6.9
II	8.0	7.2	7.5	7.5	6.5	7.2
III	8.0	7.4	6.8	8.0	6.6	7.4
IV	7.8	7.5	7.0	8.4	6.5	6.5
V	7.7	7.5	6.5	7.6	6.7	6.5
Average	8.0	7.5	7.1	8.0	6.6	6.9

## COLOR OF THE SOILS.

Comparisons of the color of the 180 foot-samples, both in an air-dry and in a moist condition, were made. For this purpose 25 gm. samples were placed in small porcelain dishes and then arranged in order of color with the darkest sample in the group at one end and the lightest-colored in that at the other. After various trials the final comparisons were made after moistening the soils by the addition to each of 10 c.c. water, covering the dish to prevent evaporation, allowing it to stand over night and making the comparison on the following day. As it was thought that the results would be more satisfactory if the soils were all brought into a definite moisture relation to their maximum water-holding capacity, as shown in the field, a number of the samples were placed in contact with a large mass of soil at its hygroscopic coefficient, treated with 50 per cent of their weight of water and allowed to stand, protected from evaporation, until water had practically ceased to be lost from them. As this method gave results no more promising than those obtained in the more expeditious manner described above, general use of the former was not made. We were able to distinguish only eight shades of color. The ranking of each sample is indicated in Table XIV. Thus that given "1" belongs to the darkest colored group, and that given "8" to the lightest. When only samples from the same area or from similar areas were compared the results obtained were fairly satisfactory, there being no difficulty in deciding which was the darker of two, if they were at all distinguishable. Thus the Wauneta and McCook samples were directly comparable, and these could, although less satisfactorily, be compared with those from Holdrege and Hastings; but the Weeping Water and Lincoln subsoils samples, while comparable with one another, could not be satisfactorily compared with the subsoils from the other areas. The difficulty is to be attributed to coloring matters other than humus, the high content of calcium carbonate in the western soils and the large amount of ferric oxide in the eastern affecting the shade produced by the ammonia-soluble black substances.

It will be seen that in general the colors bear somewhat the same relation to one another as the percentages of humus determined colorimetrically, but not as those obtained by the gravimetric method. The depth of color of a soil or subsoil is a fairly satisfactory index as to the relative amount of ammonia-soluble dark-colored organic matter present, provided other coloring matters do not occur in sufficient amounts to affect it, but as this pigment in the subsoil bears no definite relation to either the total nitrogen or the organic carbon, the color of the subsoils does not serve as a reliable index of the relative amounts of either of these constituents.

TABLE XIV.

THE RELATIVE SHADE OF COLOR OF THE SAMPLES FROM THE DIFFERENT FIELDS. 1 INDICATES THE DARKEST AND 8 THE LIGHTEST COLORED.

## WAUNETA.

Depth Ft.	Field I	Field II	Field III	Field IV	Field V	Average
1	2	2	2	2	2	2
2	3	3	2	2	3	3
3	4	2	3	3	5	3
4	6	3	4	5	7	5
5	7	6	4	6	7	6
6	8	7	4	7	8	7

## McCOOK.

1	3	2	2	2	2	2
2	4	3	4	3	6	4
3	8	4	7	6	7	6
4	8	5	8	8	7	7
5	8	7	7	8	8	8
6	8	7	8	8	8	8

## HOLDREGE.

1	1	1	1	1	1	1
2	2	3	3	3	3	3
3	5	5	5	5	5	5
4	8	8	6	8	6	7
5	8	8	8	8	8	8
6	8	8	8	8	8	8

## HASTINGS

1	1	1	1	1	1	1
2	3	3	4	3	3	3
3	6	6	6	5	6	6
4	6	8	8	8	8	8
5	8	8	8	8	8	8
6	8	8	8	8	8	8

## LINCOLN.

1	1	1	1	1	1	1
2	2	4	5	4	2	3
3	6	8	8	6	6	7
4	8	8	8	8	8	8
5	8	8	8	8	8	8
6	8	8	8	8	8	8

## WEEPING WATER.

1	1	1	1	1	1	1
2	4	4	4	3	4	4
3	8	8	8	6	8	8
4	8	8	8	8	8	8
5	8	8	8	8	8	8
6	8	8	8	8	8	8

As after complete extraction with ammonium or alkaline hydroxide the residue from the surface soil more closely resembles that from the subsoil, the original difference in color is to be attributed largely to the soluble pigment. The formation of this, which is probably a product of bacterial action, is favored to a much greater depth in the drier, better aerated subsoils of the western areas (fig. 3), which would suggest that it is produced by aerobic organisms.

#### COMPARISON WITH CHERNOZEM SOILS.

The thickness of the dark surface layer of the typical Russian Chernozem soils, according to Kossowitch (9, p. 284), is usually between 28 and 40 inches, in some places exceeding 60 and in others falling below 16, about half of this being occupied by the upper uniformly colored darker portion, the lower half passing irregularly into the light colored subsoil. While the soils of the Nebraska portion of the Transition Region show a uniformly colored upper portion and a lower portion gradually shading off into the lighter colored subsoil, the former, even in the most easterly areas, would appear to be lighter colored and shallower than the typical Chernozem, and in the western areas the soils seem to resemble more closely the Russian chestnut, or chocolate-colored soils found near the drier borders of the Chernozem zone. We find a great thickness of the dark layer only in the valleys, where it frequently extends to 3 and occasionally to 9 feet.

In the loess of the transition region we have found no dark-colored layer in the subsoil at some distance from the surface, such as is frequently found in the Russian Chernozem and there regarded as the remains of a former surface soil buried by a later deposition of loess. Near Madrid, not far from the western border of the loess in Nebraska, but on residual soil, we have found the dark soil uniform in color to a depth of seven feet, it occurring on the northeast slopes of hills as though formed by the southwest winds steadily depositing dust among the prairie plants.

Another common feature of the Chernozem, the uniformly dark-colored tongues and veins extending downward from the surface layer, seems to be entirely absent from these loess soils. In other studies one of us has encountered these in typical development in the heavier soils of the Red River Valley, as around Winnipeg. They also are found near Indian Head in Saskatchewan (5).

Data on the *matière noire* content of Russian soil are not available, the *humus* reported by Russian investigators being the same as our "organic matter of the soil," determined by combustion.

## COMPARISON WITH ARID SOILS.

It is of interest to compare the humus content of the semi-arid soils from the most westerly two areas with that of the arid soils of the regions of winter rains. Loughridge (10) has recently reported the humus in a large number of columns of California soils, most of these being taken to a depth of 12 feet and the humus determined by the Hilgard method. In general the humus is low in the surface foot and decreases gradually downward. Neither in amount nor in distribution does it appear to differ markedly from that in the semi-arid areas of this study. Table XV shows the similarity. We have no data showing to what extent the subsoils resemble one another in color, but the surface soils of the semi-arid areas are much darker in color than those of the California valleys.

TABLE XV.

COMPARISON OF AMOUNT OF HUMUS IN THE SEMI-ARID TRANSITION SOILS WITH THAT IN THE ARID CALIFORNIA SOILS REPORTED BY LOUGHRIDGE. THE HUMUS IN BOTH CASES WAS DETERMINED GRAVIMETRICALLY.

Depth Foot	Wauneta Av. 5 Fields %	McCook Av. 5 Fields %	Sacramento Valley Av. 18 Columns %	San Joaquin Valley Av. 23 Columns %
1	1.02	1.15	1.04	.80
2	.65	.62	.75	.51
3	.48	.35	.58	.37
4	.34	.31	.45	.25
5	.26	.27	.36	.23
6	.26	.27	.32	.17
Average	.50	.49	.58	.39

## SUMMARY.

The loess soils studied represent six one-foot sections from the surface downward, and the twelve one-inch sections of the surface foot, from five virgin prairie fields in each of six so-called "areas" in Nebraska, located between the Missouri River and the western limit of the loess, a distance of more than 300 miles, in which, while the temperature conditions, wind velocity, and relative humidity of the air are quite uniform there is a great range in the aridity, the mean annual precipitation decreasing from more than 30 inches in the east to less than 20 in the west, while the rate of evaporation from a free water surface during the six months, April to September, increases from 36 to 45 inches.

The gravimetric method for the determination of humus (*matière noire*) was found in the case of the subsoils to fail to indicate the relative amounts of ammonia-soluble, dark-colored organic matter present. A colorimetric method is preferable for the subsoils; in the case of the surface soils it is at least fairly satisfactory for the determination of the whole of the ammonia-soluble organic matter.

Within the surface foot the humus decreases from the first to the twelfth inch and from east to west. The rate of decrease downward is independent of the degree of aridity. In the second foot the decrease from east to west is less marked than in the first, while in the still lower levels the humus as determined gravimetrically, shows no distinct change from east to west.

No marked differences in the percentage of nitrogen in the humus was to be found between the soils from the most humid and those from the most arid parts of the region.

The soluble pigment in the surface foot was found to decrease in passing from east to west while that in the third to sixth foot increases. A relatively low amount in the surface foot with a relatively high content in the subsoil characterizes the soils from the more arid portion of the region.

The colors of the soil and subsoils agree in general with the amounts of soluble pigment found by the colorimetric method. Comparisons of the color were difficult in the case of the subsoils on account of the presence of varying amounts of coloring matters other than the soluble pigment, which causes the difference in color between surface soil and subsoil.

The color of the subsoil, like its content of soluble pigment, does not serve as an index of either the total nitrogen, the organic carbon or the ammonia-soluble organic matter present.

The color of the soils in the western areas is lighter, and in all the areas the dark-colored surface layer is shallower, than in the typical Russian Chernozem. Buried soil surfaces as well as the dark tongues and veins, common in the Russian Chernozem, appear to be absent in the loess of the Nebraska portion of the Transition Region.

Gravimetric determinations show the humus of the soils of the western semi-arid areas to be similar in amount and in distribution to that of typical arid California soils.

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# CAN SOIL BE STERILIZED WITHOUT RADICAL ALTERATION?<sup>1</sup>

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The widespread adoption of soil as a culture medium in preference to solutions in the study of soil biology problems involves a fundamental difficulty, which merits more attention than is at present being bestowed upon it. In a word, the sterilization of soil, whether by heat or antiseptics, induces certain profound changes in the chemical and physical constitution of the soil. Since the guiding motive in the use of soil as a culture medium is to obtain results which may be correlated more closely with actual field conditions, that purpose is in a measure defeated, so long as the methods of sterilization, as commonly practiced, are drastic in their effects. A review of the literature (4) failing to offer any adequate solution, a preliminary investigation was undertaken in an effort to devise some method whereby the soil might be rendered sterile with a minimum amount of alteration. Experimentation was conducted along the following lines: 1. The intermittent sterilization of soil by dry heat; 2. The relative sterilizing efficiency of various chemical substances used as soil antiseptics; 3. Volatile antiseptics applied in partial vacuum; and 4. Volatile antiseptics applied under pressure at 80° C.

## THE INTERMITTENT STERILIZATION OF SOIL BY DRY HEAT.

It has been pointed out by Russell and Hutchinson (11, 12), Pickering (8, 9), Schreiner (13), Seaver and Clark (14), and others, that heating the soil causes a distinct change in its chemical composition. Various temperatures from 60° to 170° C. have been employed with the inevitable result of an alteration in the constitution of the soil. Pickering (9) states, however, that heating at 82° C. does not cause a much greater production of toxins than heating at 50° or 60° C., consequently it was deemed advisable to use this temperature in an attempt to sterilize the soil completely. From the work of Russell and Hutchinson (11, 12), as well as that of Cunningham and Löhnis (2), it seemed valid to conclude that this degree of heat would be sufficient to kill all the living protozoa and most of the bacteria. Likewise, the cysts of protozoa were killed at 72° C. in solutions (which would represent about 60° to 65° C. in the soil). Moist heat is known to be more efficient in its destructive action than dry heat, but the laboratory facilities did not permit the use of the former.

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In view of the fact that the spores of bacteria or fungi might resist 82° C., it seemed desirable to follow a well-known bacteriological procedure and sterilize intermittently for a number of days at the same temperature. In this way the spores present would have ample opportunity to germinate, and the living forms would immediately succumb to the temperature which the spores might have been able to resist. Likewise, by this method the production of toxins, and the alteration of the chemical composition of the soil was reduced to a minimum, while the biological factors suffered decimation.

A point of considerable importance which had been attacked by Richter (10) and Koch (3), namely, the difference in the effect of sterilization upon air-dry and moist soil, was included in this investigation.

The method of procedure was as follows, using Penn Clay Loam soil, a chemical analysis of which is given in Table I. Because of the fact that it is more difficult to sterilize a heavier than a lighter soil, the results obtained by using Penn Clay Loam would thus be more exacting, than if a sandier soil were employed.

TABLE I.  
CHEMICAL ANALYSIS OF PENN CLAY LOAM.  
(HCL sp. gr. 1.115)

	Per Cent		Per Cent
Insoluble Matter . . . . .	78.2820	P <sub>2</sub> O <sub>5</sub> . . . . .	.1282
K <sub>2</sub> O . . . . .	.5496	SO <sub>2</sub> . . . . .	.0497
Na <sub>2</sub> O . . . . .	.1790	Volatile Matter . . . . .	8.9200
CaO . . . . .	.3962	Total Nitrogen . . . . .	.1722
MgO . . . . .	.9041	Total Phosphoric Acid . . . . .	.1340
Fe <sub>2</sub> O <sub>3</sub> . . . . .	4.1900	Total Potash . . . . .	2.1700
Al <sub>2</sub> O <sub>3</sub> . . . . .	6.3168	Total Carbon . . . . .	2.4500

Fifty-gram portions of soil were placed in cotton-plugged 200 c.c. Erlenmeyer flasks. One series of ten flasks containing air-dry soil (having 4.5 per cent water), the other series of ten flasks containing 25 per cent of water (calculated on the water-free basis), which was equivalent to 60 per cent of the moisture-holding capacity of the soil.

Both series of flasks were incubated at 22° C. for 24 hours to allow excystation of protozoa in moist soil and to ensure the presence of vegetative forms of bacteria. (It might be noted, however, that a three-day incubation would have been preferable.) The flasks were then placed in a hot-air oven and heated until the constant temperature of the soil registered 82° C. for one hour. After this treatment all the flasks were again placed in the incubator at 22° C. for 24 hours, and two flasks of each series were taken for bacterial counts on Lipman and Brown's (5) "synthetic" agar. The counts were made in triplicate. The plates were counted after 3 days had elapsed.

The process outlined above was repeated for 5 successive days, two flasks of each series being removed each day and bacterial counts made on the results of each day's heating. On the last day, the soil was incubated for 48 hours instead of 24 to make doubly certain of having all the biological forms in the soil in the active living state. Also the plates were incubated for 7 instead of 3 days.

TABLE II.  
INTERMITTENT STERILIZATION BY DRY HEAT AT 82° C.

MOIST SOIL (PENN CLAY LOAM) 25% H<sub>2</sub>O.

Treatment	Bacterial Count Millions per gm. Soil		Total Water-Solu- ble Solids in gm.		Organic Solids in gm.		Inorganic Solids in gm.	
	Dup.	Av.	Dup.	Av.	Dup.	Av.	Dup.	Av.
Check .....	48.000 47.500 12.300	47.750	.0216 .0224 .0304	.0220	.0108 .0132 .0164	.0120	.0108 .0092 .0140	.0100
After 1st Day's Heating	12.400 0.711	12.350	.0316 .0320	.0310	.0180 .0176	.0172	.0136 .0144	.0138
After 2nd Day's Heating	0.632 0.075	0.672	.0316 .0282	.0318	.0172 .0118	.0174	.0144 .0164	.0144
After 3rd Day's Heating	0.067 0.037	0.071	.0296 .0310	.0289	.0184 .0170	.0151	.0112 .0140	.0138
After 4th Day's Heating	0.046 0.001	0.042	.0325 .0322	.0318	.0170 .0173	.0176	.0145 .0149	.0143
After 5th Day's Heating	0.002	0.005	.0320	.0321	.0167	.0170	.0153	.0151

AIR-DRY SOIL (PENN CLAY LOAM) 4.5% H<sub>2</sub>O.

Check .....	45.000 42.000 52.500	43.050	.0216 .0224 .0200	.0220	.0108 .0132 .0164	.0120	.0108 .0092 .0036	.0100
After 1st Day's Heating	50.200 11.300	51.350	.0212 .0332	.0206	.0164 .0176	.0164	— .0048 .0166	— .0042
After 2nd Day's Heating	11.800 9.250	11.500	.0320 .0260	.0326	.0164 .0172	.0170	— .0156 .0084	— .0161
After 3rd Day's Heating	9.660 3.525	9.400	.0270 .0237	.0265	.0180 .0121	.0176	— .0092 .0116	— .0088
After 4th Day's Heating	4.400 3.424	3.962	— .0220 .0228	— .0229	.0112 .0075	.0116	— .0108 .0153	— .0112
After 5th Day's Heating	3.500	3.462	.0271	.0250	.0122	.0093	.0149	.0151
Moist heat at 120° C. for 15 min. at 15 lbs. pres're	Sterile		.1810 .1800		.0900 .1005		.0795 .0910	
				.1805		.0953		.0853

Treatment in Moist Soil (25 % H <sub>2</sub> O)	Ammonia Release Average in grams
Check .....	.0014
After 1st Day's Heating.....	.0021
After 2nd Day's Heating.....	.0019
After 3rd Day's Heating.....	.0019
After 4th Day's Heating.....	.0019
After 5th Day's Heating.....	.0019

The results are recorded in Table II. All the protozoa were killed after the initial treatment by heating to 82° C. for one hour. Löhnis' (2) soil extract was employed as a medium for determining the presence of protozoa, 100 c.c. being inoculated with 50 gm. of soil, and a microscopical examination made for 5 successive days. Fungi, however, persisted throughout the experiment; species of *Penicillium* and *Mucor* being recognized.

An examination of the results of the bacterial counts represented in figure 1 reveals the fact that in moist soil the numbers of bacteria decrease successively, in a remarkable manner from 47,750,000 per gram on the first day to 1,500 on the last day. On the other hand, however, there is an initial depression of bacterial numbers exhibited in the air-dry soil, followed by a gradual decrease in the numbers of bacteria on the subsequent days, which is substantially less effective than the decrease operating in the moist soil. A variation is noted in the bacterial counts on air-dry soil on the second day, for instead of a decrease there is an increase which may be ascribed to the immediate utilization of some nutrients becoming more available by the first day's heating.

It may be observed, parenthetically, that the soil was incubated for 48 hours instead of 24 on the last day, thus enabling the bacteria to multiply to a considerable extent after removal from the sterilizer and magnifying the bacterial count accordingly. Consequently, the number recorded is not a fair index of the actual number of bacteria present in the soil immediately after heating, but represents an addition over and above that amount.

Further, the plates were incubated for 7 days instead of 3 days for the purpose of ascertaining whether the number of colonies increased to any appreciable extent after 3 days. A negligible increase was noted.

Therefore, it is evident from the above results, that the intermittent sterilization of moist soil by dry heat is decidedly more efficacious in reducing the bacterial numbers than the same treatment with air-dry soil.

For the purpose of determining the amount of increase of the water-soluble constituents of the soil as a result of intermittent sterilization by dry heat, the following method was employed.

Fifty grams of the same kind of soil were heated under the same conditions as previously and then vigorously shaken with distilled water until the leachings made up a volume of 200 c.c. An aliquot portion of 50 c.c. was evaporated to dryness on a water-bath, dried in a hot-air oven at 108° C. for one hour (according to the method described by Seaver and Clark) (5), weighed, ignited, and then weighed again. The first weight represents the total solids, the final weight represents the inorganic constituents, and the difference between the two weights represents the organic constituents.

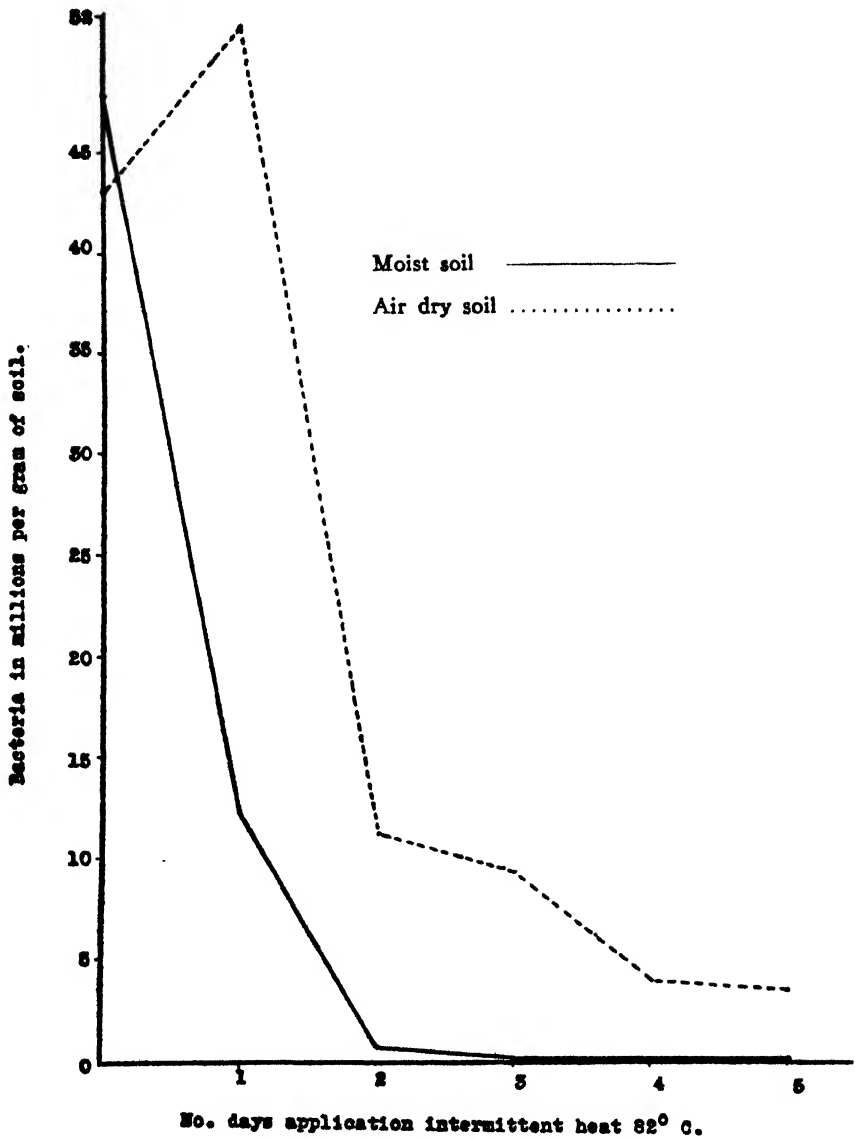


Fig. 1. Diagram showing the effect of intermittent heat for 5 days at 82° C. upon the numbers of bacteria in moist soil and in air-dry soil.

It is seen from Table II that sterilization under these conditions increases the amount of total solids 45.91 per cent; and that after the initial heating the amount of total solids does not increase within experimental error.

It is interesting to note the comparison between the increase in the amount of water-soluble solids as a result of intermittently dry heat at 82° C. and the increase due to subjecting the soil to steam at 120° C. under 15 pounds pressure for 15 minutes, which is the usual laboratory method employed in soil biology investigations. The latter method is responsible for almost sixteen times the amount liberated by subjection to intermittent dry heat. The ammonia released by the sterilizing treatment at 82° C. is greatest after the first day and remains practically constant thereafter.

Considerable variation is noted in the amount of total solids in the air-dry soil, and this discrepancy may be accounted for by the fact that the heat was not able to permeate uniformly throughout the soil in a dry state.

In summarizing the foregoing results, the following points are to be noted:

1. Intermittent partial sterilization at 82° C. kills the greater portion of the bacterial population, as indicated by growth on Lipman and Brown's synthetic agar.
2. The treatment kills all the protozoa in the soil, as indicated by their non-appearance in Löhnis' soil extract medium.
3. Fungi persist throughout the experiment, as indicated by their presence on the culture media.
4. The sterilization treatment increases the total solids in the soil about 46 per cent, thereby altering the chemical composition of the soil and changing it as a medium for biological activity, but only one-sixteenth as much as by the common method of steam sterilization.
5. Where the time-element is of considerable importance the above method is undesirable.

#### THE RELATIVE STERILIZING EFFICIENCY OF VARIOUS CHEMICAL SUBSTANCES USED AS SOIL ANTISEPTICS.

The use of various chemical substances for the purpose of sterilizing soil has long been practiced not only in the laboratory, but in the greenhouse and under field conditions as well. Russell and Hutchinson (12), and recently Buddin (1), have made an extensive survey of substances which would prove adequate in presenting the so-called "partial sterilization" phenomena. The results obtained by the former investigators indicate that volatile antiseptics in quantities of one per cent are as efficient as when used in greater amounts. For this reason one per cent (on the

basis of 100 gm. of air-dry soil) of the following volatile antiseptics were employed: ethyl alcohol ( $C_2H_5OH$ ), ethyl ether [ $(C_2H_5)_2O$ ], hydrogen peroxide ( $H_2O_2$ ), toluene ( $C_6H_5CH_3$ ), carbon bisulfid ( $CS_2$ ), and chloroform ( $CHCl_3$ ).

The procedure of this experiment was as follows: 100-gm. portions of Penn Clay Loam (passing a 20-mesh sieve) were placed in cotton-plugged 200 c.c. Erlenmeyer flasks. The soil was then brought to optimum moisture content by the addition of 25 c.c. of sterile tap water. One per cent quantities of the above-mentioned volatile antiseptics were then added to the soil. Each treatment was carried out in duplicate. In the case of the addition of hydrogen peroxide, 5 c.c. of concentrated solution were previously added to 20 c.c. of sterile tap water and the mixture used to bring the soil to the optimum moisture content. Following the addition of the antiseptics the flasks were sealed with corks which had previously been steeped in paraffine. The antiseptics were allowed to remain in contact with the soil for 3 days at room temperature. At the expiration of this time, the paraffine corks were removed from the flasks, and sterile cotton plugs substituted. In their work on partial sterilization, Russell and Hutchinson (11) removed the volatile antiseptics from the soil after treatment, by exposing the latter to the air for a time. It would be practically impossible to accomplish this under sterile conditions, consequently the following apparatus, illustrated in figure 2, was devised.

The vacuum chamber (A) is a strong metal tank (in this case a fire extinguisher emptied of its contents was employed), which is connected with a water-pump (F) for exhausting the air from the chamber. Another connection is made with the barometer (I), which consists of a graduated glass tube inverted in a bottle of mercury. In a subsequent portion of this paper mention will be made of the volatilization of antiseptics by immersion of a bottle of antiseptic (D) in boiling water (E). This connection is likewise indicated in the diagram. Stop-cocks were placed at the points marked C. It might be added parenthetically that the capacity of the vacuum chamber (A) in which the flasks (B) were placed is 11,500 c.c.

In the experiment under discussion the flasks (B) were placed in the vacuum chamber (A) and the latter exhausted (G) to one-half inch mercury pressure (F). After remaining in the chamber for one-half hour, the flasks were removed and the soils plated out on Lipman and Brown's (5) synthetic agar for bacterial counts in the usual manner. The total water-soluble solids were determined by the method previously described under the sterilization of soil by intermittent dry heat. An examination of the soils for the presence of any antiseptics, revealed the fact that toluene was the only substance remaining in any appreciable quantity in the soil after such treatment.



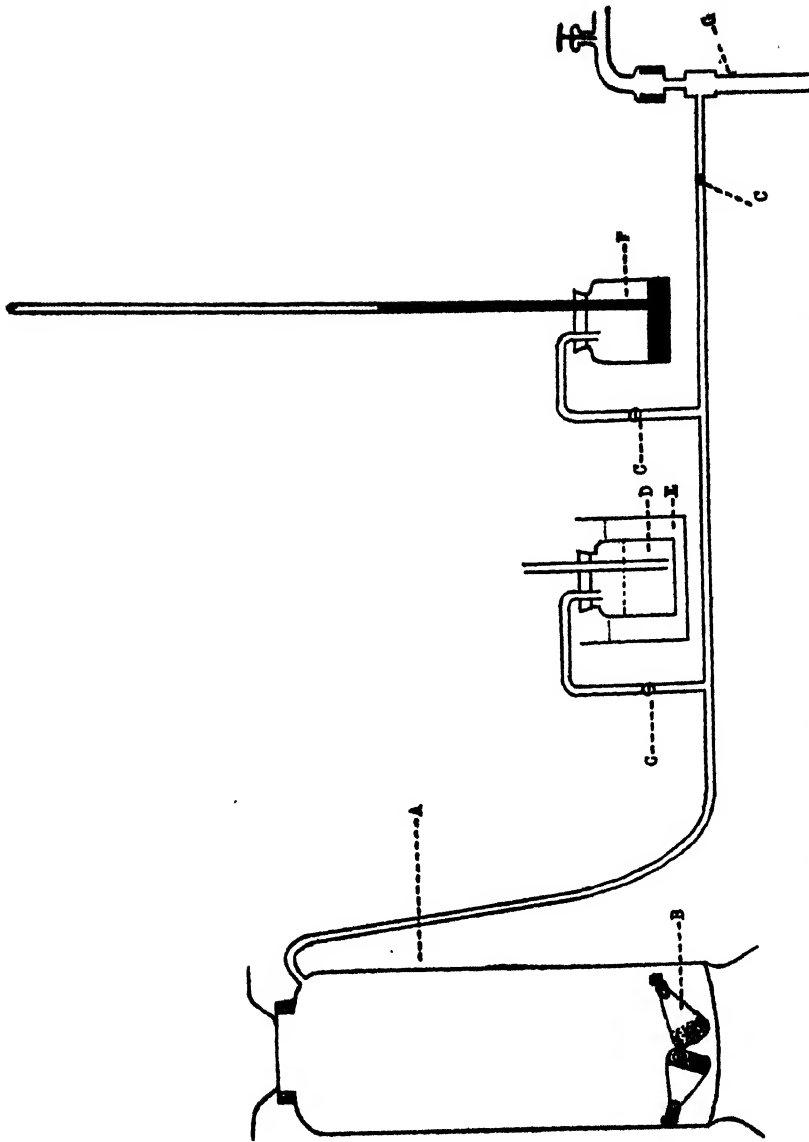


Fig. 2. Diagram of apparatus used in experiment on sterilizing soil with various antiseptics.

It will be seen from the results in Table III that this method of applying antiseptics is not sufficiently efficient to justify its use as a practical means of soil sterilization. Of the substances employed, chloroform,

TABLE III.  
THE RELATIVE EFFICIENCY OF VOLATILE ANTISEPTICS (1%)  
IN SOIL STERILIZATION.

MOIST SOIL.

Lab. No.	Antiseptic	Bacteria in millions per gm. of soil	Average	Total Water-soluble Solids, Av. in gm.
210	Original Soil .....		47.75	.0220
298	Check .....	44.50		
299	Check .....	51.00	47.75	.0902
334	Alcohol (Ethyl) .....	44.50		
335	Alcohol (Ethyl) .....	42.50	43.50	.1045
300	Ether (Ethyl) .....	41.50		
301	Ether (Ethyl) .....	37.00	39.25	.1090
322	Hydrogen Peroxide .....	41.00		
323	Hydrogen Peroxide .....	26.50	33.25	.0990
310	Toluene .....	11.50		
311	Toluene .....	27.00	19.25	.0947
328	Carbon Bisulfid .....	13.50		
329	Carbon Bisulfid .....	5.50	9.50	.1097
316	Chloroform .....	9.00		
317	Chloroform .....	4.50	6.75	.0915

carbon bisulfid, and toluene, in the order named, were effective in decimating the bacterial flora; chloroform being responsible for a decrease of 86 per cent of the original bacterial content. Furthermore, chloroform caused the least alteration in the chemical constitution of the soil, as indicated by the total water-soluble solids, which in this case amounted to something more than three times the original quantity present.

Under the conditions of the experiment, volatile antiseptics when applied to moist soil in amounts of 1 per cent were not efficient as sterilizing agents. Acting upon the assumption that dry soil is more difficult to sterilize than moist soil, the plan of repeating this experiment with air-dry soil was abandoned.

THE RELATIVE EFFICIENCY OF VOLATILE ANTISEPTICS APPLIED AS  
VAPOR IN PARTIAL VACUUM.

The problem of equal distribution throughout the soil mass is a serious one in the application of volatile antiseptics in such small amounts as 1 per cent. Obviously, moist soil is superior to dry soil in facilitating uniform distribution; nevertheless, it would be of advantage to increase the efficiency even of the former, in this direction. With this in view, the vacuum chamber (figure 2) previously described was adapted to the needs of the following experiment.

The principle involved is, in effect, the application of volatile antiseptics *in vacuo*, thus obtaining an intimate and uniform mixture of soil and chemical. A further modification has been introduced which is based upon the fundamental physical law that gases diffuse more rapidly than liquids; namely, the volatile antiseptics are applied in the form of vapor rather than in the usual liquid state. This combination of the two principal factors of vapor and vacuum is effected by means of the apparatus devised; ergo, a more uniform distribution of the antiseptics in the soil is achieved.

As in former cases, 100-gm. portions of soil were placed in Erlenmeyer flasks (plugged with cotton). In the first series of treatments air-dry soil was employed, whereas in the second series moist soil was used. The volatile antiseptics tested were: osmic acid, ethyl ether, carbon bisulfid, toluene, and ethyl alcohol.

The method of procedure was as follows: The flasks containing soil were placed in the vacuum chamber (A—see figure 2), and the latter exhausted to one-half inch of mercury pressure (F). A bottle containing the antiseptic (D) was immersed in boiling water (E), in order that the application might be made in the form of vapor. By means of a direct connection the vapor entered the vacuum chamber. The flasks were allowed to remain in this antiseptic atmosphere for one and one-half hours, thereby allowing the soil to take up as much vapor as it could. The vacuum chamber (A) was kept in close proximity to a source of heat in order that the antiseptic might remain in a volatile state. During the course of the various treatments pressure was developed within the vacuum chamber as a result of the antiseptics passing from the liquid to the gaseous state. Indirectly, this development of pressure may be considered as being indicative of the fact that the antiseptic vapor has saturated the pore spaces of the soil, which had previously been exhausted.

After the antiseptic vapor had been allowed to remain in intimate contact with the soil for one and one-half hours, the vacuum chamber was again exhausted to one-half inch of mercury pressure in order that the antiseptics might be removed from the soil. Air, which had been rendered sterile by filtering through cotton, was then admitted to the vacuum chamber, and the soils plated immediately for the bacterial count. The process outlined above was repeated on each of three successive days and the total water-soluble solids determined at the conclusion of that period.

It will be observed from the results recorded in Table IV that carbon bisulfid, toluene, and ethyl alcohol, in the order named, were quite effective in decreasing the bacterial content of the air-dry soil, as is evidenced by the fact that the number of bacteria fell from 43,000,000 to 160,000 in the case of the carbon bisulfid and to 250,000 and 330,000 as a result

of treatment with toluene and ethyl alcohol, respectively. In effect, this is a decrease of more than 99 per cent of the original soil flora.

TABLE IV.  
THE RELATIVE EFFICIENCY OF VOLATILE ANTISEPTICS APPLIED AS VAPOR  
IN PARTIAL VACUUM.

AIR-DRY SOIL.

Antiseptic	Bacteria in millions per gram of soil						Total Water-Soluble Solids Av. in gm.	Pressure developed during treatment
	1st Day	Av.	2d Day	Av.	3d Day	Av.		
Original Soil .....		43.05		43.05		43.05	.0220	
Check .....	10.00		8.80		4.80			
Check .....	9.00	9.50	8.00	8.40	4.20	4.50	.0890	29 in. Hg.
Osmic Acid .....	7.85		6.50		5.00			
Osmic Acid .....	7.95	7.90	10.00	8.75	2.70	3.85	.0895	28 " "
Ethyl Ether .....	3.60		3.40		2.75			
Ethyl Ether .....	3.50	3.55	2.70	3.05	2.00	2.37	.0975	20 " "
Carbon Bisulfid .....	3.50		0.80		0.12			
Carbon Bisulfid .....	lost	3.50	0.80	0.80	0.20	0.16	.1190	20 " "
Toluene .....	6.90		5.10		0.29			
Toluene .....	8.00	7.45	lost	5.10	0.21	0.25	.1190	12 " "
Ethyl Alcohol .....	2.20		2.50		0.30			
Ethyl Alcohol .....	2.10	2.15	2.70	2.60	0.36	0.33	.1195	4½" "

In considering the decrease in bacterial numbers from day to day as a result of treatment, in the case of carbon bisulfid there is a decrease on the second day 75 per cent of the number of bacteria present on the first day, followed by a decrease on the third day of 80 per cent of the number present on the second day. There is, then, good reason to believe that if the treatment were prolonged over a greater period of time, the number of bacteria might be still further reduced. The operation of the time-factor, however, must be regarded as a distinct limitation upon any method of protracted duration. With toluene and ethyl alcohol there is likewise a striking reduction in numbers from the second to the third day. It will be noted in this experiment that osmic acid proved to be unsuccessful as a sterilizing agent for soil. Ethyl ether, as well, was found to be inefficient.

With regard to the total water-soluble solids it will be observed that there is, generally speaking, a fourfold increase. No correlation can be established between the sterilizing efficiency of the antiseptics in question, and the pressure developed by them in the gaseous state during treatment, although the data may not be altogether without interest.

It may be seen from Table V that the final results for moist soil are similar to those obtained where dry soil was employed, with the exception of ethyl ether which yields the largest reduction of bacterial num-

bers. Carbon bisulfid, toluene and ethyl alcohol again manifest a fair degree of efficiency, although their effectiveness is not as great in moist soil as in dry soil. This may possibly be explained on the grounds that in the case of the moist soil the bacteria had ample opportunity to multiply in the 24-hour interval between sterilizations; or, on the other hand, it is not impossible to suppose that the moist soil offers greater resistance to the penetration of the antiseptic vapors than the dry soil. The reduction in bacterial numbers on successive days is noteworthy, although not quite so marked as in the treatment of dry soil.

TABLE V.

THE RELATIVE EFFICIENCY OF VOLATILE ANTISEPTICS APPLIED AS VAPOR IN PARTIAL VACUUM.

## MOIST SOIL.

Antiseptic	Bacteria in millions per gram of soil						Total Water-Soluble Solids Av. in gm.	Pressure developed during treatment
	1st Day	Av.	2d Day	Av.	3d Day	Av.		
Original Soil .....		47.75		47.75		47.75	.0220	
Check .....	8.30		5.30		3.90			
Check .....	8.00	8.15	4.80	5.05	3.60	3.75	.0895	29 in. Hg.
Osmic Acid .....	3.50		4.00		4.70			
Osmic Acid .....	2.70	3.10	4.90	4.45	2.00	3.35	.0860	28 " "
Ethyl Ether .....	3.00		1.10		0.15			
Ethyl Ether .....	4.50	3.75	0.92	1.01	0.20	0.17	.1215	20 " "
Carbon Bisulfid .....	10.00		0.70		0.50			
Carbon Bisulfid .....	13.00	11.50	0.70	0.70	0.46	0.48	.1145	20 " "
Toluene .....	4.30		1.80		1.60			
Toluene .....	4.40	4.35	0.70	1.25	0.63	1.11	.0950	12 " "
Ethyl Alcohol .....	6.70		2.20		1.00			
Ethyl Alcohol .....	6.80	6.75	2.70	2.45	1.20	1.10	.1190	4 3/4 " "

The superiority of osmic acid over the check treatment is virtually insignificant. The treatments employed were responsible for an increase in total water-soluble solids, as formerly, of approximately four times the original amount. From the data presented in Tables IV and V it may be inferred that volatile antiseptics applied as vapor in partial vacuum are relatively efficient in the sterilization of soil. This method, if sufficiently prolonged, might render the soil totally sterile, yet the increase in water-soluble constituents must also be considered, and with this point in view it is evident that the soil is compelled to undergo some alteration.

## THE RELATIVE EFFICIENCY OF VOLATILE ANTISEPTICS WHEN APPLIED UNDER HEAT AND PRESSURE.

Having obtained results of a somewhat encouraging nature from the use of intermittent heat at 82° C., it was deemed advisable to combine this method with a still further modification of the application of volatile antiseptics, namely, employing the latter under pressure at 80° C. for three successive days. It was observed in the preceding experiment that when antiseptics were vaporized in an air-tight chamber they automatically developed pressure.

The method of procedure was as follows: A vertical steam pressure autoclave (American Standard), commonly employed in the bacteriological laboratory for sterilization, was filled to within 5 inches of the top with water and the temperature raised to 80° C. An agate pan was floated on the surface of the water and the Erlenmeyer flasks (plugged with cotton) containing moist soil were placed therein. One hundred cubic centimeters of the volatile antiseptics under investigation were poured into the agate pan. The lid of the autoclave was then quickly clamped down and the check valve closed upon the appearance of the vapor. As a result of the vaporization of the antiseptic, pressure was developed. (It is assumed throughout this work with volatile antiseptics that the usual precautionary methods are observed, and all flames in the vicinity of the vapors extinguished.) The flasks were allowed to remain in the autoclave for one hour,<sup>1</sup> after which they were removed to the vacuum chamber, which was exhausted to one-half inch mercury pressure. After remaining in the chamber for one-half hour to allow sufficient time for the removal of the antiseptic, sterile air was admitted and the soils plated immediately for bacterial counts. The entire 100 gm. of soil were taken up with one liter of sterile water and the ordinary dilutions employed. It was impracticable to use alcohol in this experiment for the reason that it would remain in solution in the water.

From the results in Table VI it will be noted that this method of treatment was fairly effective in depopulating the bacterial flora of the soil. The decrease, in general, approximated 98 per cent. Carbon bisulfid was the only antiseptic employed that proved superior to the check treatment. In this case a possible correlation might be obtained between the pressure developed during treatment and the effectiveness of the sterilizing agent. Thus carbon bisulfid developed a pressure of 20 pounds, whereas ethyl ether, which proved least efficient in sterilization, developed only 6 pounds of pressure. Carbon tetrachloride, with only 5 pounds pressure, is an exception to such a generalization.

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<sup>1</sup> During the period of treatment the temperature fell, on the average, ten degrees.

TABLE VI.  
THE RELATIVE EFFICIENCY OF VOLATILE ANTISEPTICS APPLIED UNDER  
HEAT AND PRESSURE.

## MOIST SOIL.

Antiseptic	Bacteria in millions per gram of soil						Total Water-Soluble Solids Av. in gm.	Pressure developed during treatment
	1st Day	Av.	2d Day	Av.	3d Day	Av.		
Original Soil .....		47.75		47.75		47.75	.0220	
Check .....	10.00		1.27		0.55			
Check .....	9.00	9.50	1.17	1.22	0.65	0.60	.0885	0
Carbon Tetrachloride .....	11.50		1.17		0.45			
Carbon Tetrachloride .....	12.50	12.00	2.60	1.88	0.80	0.62	.1097	5 lbs.
Carbon Bisulfid .....	10.50		0.41		0.30			
Carbon Bisulfid .....	6.50	8.50	0.34	0.37	0.15	0.22	.1170	20 "
Ethyl Ether .....	9.50		0.96		1.55			
Ethyl Ether .....	11.50	10.50	0.75	0.85	1.30	1.42	.1102	6 "
Chloroform .....	8.50		1.50		0.95			
Chloroform .....	4.50	6.50	1.50	1.50	0.80	0.87	.1055	10 "

In the matter of total water-soluble solids, the increase is again approximately fourfold. This experiment was repeated, substituting air-dry for moist soil, with little or no increase in effectiveness of volatile antiseptics over the check treatment.

In a general comparison of the volatile antiseptics employed under the various treatments as outlined, shown in Table VII, the total decrease (in per cent) of the bacterial numbers from the original bacterial content of the soil is recorded, as well as the increase in total water-soluble solids (in per cent) over the amount originally present in the soil.

Under the conditions of this experiment it will be readily observed from the calculations represented in Table VII that intermittent dry heat at 82° C. for 5 successive days in moist soil was responsible for the greatest bacterial decrease, or 99.996 per cent. Likewise, this method caused the minimum alteration in the chemical constitution of the soil as indicated by the amount of total water-soluble solids. It is of interest to note that, whereas the above method caused a 46 per cent increase of total water-soluble solids, the ordinary moist heat sterilization at 120° C. for 15 minutes at 15 pounds pressure, which is widely used in soil biology investigations, caused an increase of 720.45 per cent. Thus it is evident that the latter method induces a radical alteration in the composition of the soil compared with the former.

Of the three other methods devised, that of applying volatile antiseptics in partial vacuum for three successive days proved somewhat superior to the application of these chemicals under heat (80° C.) and pressure for a similar period. Volatile antiseptics applied in 1 per cent. quantities in the liquid state are inefficient. Carbon bisulfid, in general,

proved to be the most efficient volatile antiseptic tested with regard to its practical value as a sterilizing agent. The three methods just mentioned caused approximately a fourfold increase in total water-soluble solids.

TABLE VII.  
COMPARISON OF THE VOLATILE ANTISEPTICS EMPLOYED UNDER VARIOUS TREATMENTS SHOWING TOTAL DECREASE (IN PER CENT) OF BACTERIAL NUMBERS FROM ORIGINAL BACTERIAL CONTENT OF THE SOIL; AND INCREASE OF TOTAL SOLIDS (IN PER CENT).

Treatment	Total decrease of bacterial numbers resulting from treatment (av.)							
	1% applied as liquid 3 days in moist soil		Partial Vacuum 3 Days				Pressure and heat (80° C.) for 3 days	
			Moist Soil		Air-Dry Soil			
	Bact. Dec. %	Total Solids Inc. %	Bact. Dec. %	Total Solids Inc. %	Bact. Dec. %	Total Solids Inc. %	Bact. Dec. %	Total Solids Inc. %
Original Soil—Ck. Treatment.		310.00	92.15	306.81	89.55	304.54	98.75	302.27
Carbon Tetrachloride .....							98.71	398.63
Carbon Bisulfid .....	80.11	394.08	99.00	420.45	99.63	440.90	99.54	431.81
Ethyl Ether .....	17.80	395.45	99.65	452.27	94.50	343.18	97.03	400.91
Chloroform .....	85.87	315.89					98.18	379.54
Osmic Acid .....			92.98	290.91	91.06	306.81		
Toluene .....	59.69	330.45	97.68	331.81	99.43	440.90		
Ethyl Alcohol .....	8.91	375.00	97.70	440.91	99.24	443.18		
Hydrogen Peroxide .....	30.37	350.00						
	Moist Soil		Air-Dry Soil					
Intermittent Dry Heat at 82° C. for 5 days .....	99.996	45.91	91.96	13.63				
Moist Heat at 120° C. for 15 min. at 15 lbs. pressure.....	Sterile	720.45						

The data presented above are preliminary in character, and although no definite conclusions can be established, several lines of investigation are indicated which might prove adequate in solving the problem of soil sterilization without radical alteration.

#### SUMMARY.

1. Under the conditions of the experiment, with Penn Clay Loam, intermittent sterilization by means of dry heat at 82° C. for 5 successive days in moist soil, almost completely decimated the bacterial flora of the soil. This was accomplished with but a slight change in the chemical constitution of the soil, as indicated by the amount of water-soluble solids. Ordinary steam sterilization under pressure causes a change sixteen times as great.

2. There is a strong indication that the application of volatile antiseptics either in partial vacuum or under a combination of heat and pressure, if repeated for more than three successive days, would achieve complete soil sterilization without involving any radical alteration in the chemical constitution of the soil.

In conclusion, it is a privilege to express an appreciation of Dr. J. G. Lipman's suggestions, ever at our disposal.



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# INCUBATION STUDIES WITH SOIL FUNGI.<sup>1</sup>

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## INTRODUCTION.

Recent investigations in the field of microbiology have shown in a measure the importance of recognizing fungi as one of the factors in soil fertility. In studying the complex problem of microörganic activities, too little attention has been directed to the possible influence of fungus forms. In fact, many of the chemical changes taking place constantly in soils have been considered almost wholly the result of bacterial life, while now we know that a large number of fungi are capable of working in some directions quite as efficiently as bacteria. Whether or not fungi have the capacity to fix nitrogen or to nitrify, cannot be definitely settled as yet, but many of them do have the power to ammonify. Their importance is thus further emphasized by the fact that they occur in such large numbers in cultivated and uncultivated soils.

Since the morphology and life history of fungi are much more complex than those of bacteria, we have reason to suspect that the development of different stages in the life of these organisms may simultaneously affect their powers to decompose organic matter; whereas the very short life cycle of bacteria renders their functions in the soil a more or less continuous process, the fact that fungi have a much longer life cycle would no doubt affect their relation to the fertility of the soil differently at successive stages of growth.

In order to learn something of the factors involved in this phase of biological activities, the present investigations were begun.

## EXPERIMENTAL.

The character of this study was such that in the very beginning the usual methods of working with soil fungi were questioned. In general, these methods were essentially the ones used in work with bacteria, and the adaptability was thereby merely assumed. It was therefore planned to carry out a few preliminary experiments in an effort to determine two important elements: first, moisture requirements; and second, the proper incubation period.

The soil used throughout was a Sassafras gravelly loam, dried blood and cottonseed meal serving as ammoniates. Three organisms were employed as follows: *Mucor plumbeus*, *Penicillium sp.*,<sup>2</sup> and *Monilia sito-*

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<sup>1</sup> Received for publication September 15, 1915.

<sup>2</sup> This organism belongs to a group of soil *Penicillia* which are at present under investigation.

*phila*. These were isolated and purified on the fungi medium noted below.<sup>1</sup> Subcultures were made in liquid media of the same composition and incubated at 25° C. until spores were produced. One-cubic centimeter portions of these cultures were used for inoculation.

### SERIES I.

#### Moisture Relationship and Incubation Period.

In this series four different amounts of moisture were tried. The organic matter, in quantities containing 100 mg. of nitrogen, was added to fifty-gram portions of soil and thoroughly mixed. The physical optimum of the mixtures was then determined (16 per cent resulting for the soil with dried blood and 17 per cent for the soil with cottonseed meal). The moisture was varied and added as noted below; 1 c.c. in excess being added to all to allow for loss during sterilization which was accomplished at 15 pounds pressure for 15 minutes. The soil portions were properly inoculated and subsequently incubated at 22° C. These were divided into three sets, a set being distilled for ammonia after six, twelve, and eighteen days respectively. *Mucor plumbeus* and *Penicillium* sp. were used for inoculation.

TABLE I.

AMMONIFICATION BY MUCOR PLUMBEUS AND PENICILLIUM SP. IN 6 DAYS.

Moisture added	Organic comp.	Check		Mucor plumbeus			Penicillium sp.		
		Ammonia N found		Ammonia N found			Ammonia N found		
		mg. N	Ave'ge	mg. N	Ave'ge	Inc. over check	mg. N	Ave'ge	Inc. over check
¼ optimum	Dried Blood	2.2		3.20			5.10		
		2.4	2.30	4.40	3.80	1.50	6.50	5.80	3.50
		2.2		5.80			8.65		
½ "	"	2.2	2.20	8.10	6.95	4.75	8.30	8.48	6.28
		2.3		14.65			10.20		
1 "	"	2.2	2.25	13.45	14.05	11.80	9.50	9.85	7.60
		2.3		12.55			9.30		
2 "	"	2.3	2.30	12.85	12.70	10.40	10.30	9.80	7.50
		2.6		8.65			3.75		
¼ optimum	Ctn Sd Meal	2.6	2.60	7.55	8.10	5.50	3.60	3.68	1.08
		2.3		12.60			3.40		
½ "	"	2.4	2.35	12.80	12.70	10.35	3.40	3.40	1.05
		2.4		13.75			3.15		
1 "	"	2.5	2.45	13.60	13.68	11.23	3.15	3.15	.70
		2.3		9.80			3.15		
2 "	"	2.3	2.30	11.60	10.70	8.40	3.45	3.30	1.00

<sup>1</sup> Medium No. II as described in "The relation of parasitic fungi to the contents of the cells of the host plants," Cook, M. T., and Taulenhaus, M. S., Del. Agr. Exp. Stat., Bul. 91, p. 11.

Table I shows that the amount of ammonia accumulation in six days by *Mucor plumbeus* from both dried blood and cottonseed meal increases from one-fourth optimum to optimum moisture and then slightly decreases. Essentially the same conditions are true with *Penicillium sp.* except that with cottonseed meal the ammonia accumulation was so little that differentiation was difficult.

TABLE II.

AMMONIFICATION BY MUCOR PLUMBEUS AND PENICILLIUM SP. IN 12 DAYS.

Moisture	Organic comp.	Check		Mucor plumbeus			Penicillium sp.		
		Ammonia N found		Ammonia N found			Ammonia N found		
		mg. N	Ave'ge	mg. N	Ave'ge	Inc. over check	mg. N	Ave'ge	Inc. over check
$\frac{1}{4}$ optimum	100 mg. N. in Dried Blood	2.3		6.60			13.15		
		2.4	2.35	6.50	6.55	4.30	16.50	14.83	12.48
		2.1		10.00			23.00		
$\frac{1}{2}$ "	"	2.8	2.45	14.00	12.00	9.55	26.20	24.60	22.15
		2.1		19.80			26.20		
1 "	"	2.6	2.35	19.70	19.75	17.15	25.10	25.65	23.30
		2.1		20.40			24.05		
2 "	"	2.2	2.15	lost	20.40	18.25	20.40	22.23	20.08
		2.4		17.50			8.00		
$\frac{1}{4}$ optimum	100 mg. N. in Ctn Sd Meal	2.8	2.60	15.70	16.60	14.00	8.80	8.40	5.80
		2.3		20.80			7.50		
$\frac{1}{2}$ "	"	3.3	2.80	21.00	20.90	18.10	12.25	9.88	7.08
		2.4		22.55			12.50		
1 "	"	2.8	2.60	21.15	21.85	19.25	lost	12.50	9.90
		2.2		18.60			12.60		
2 "	"	2.9	2.55	23.10	20.85	17.95	12.00	12.30	9.40

In Table II we find that in twelve days also the ammonia accumulation goes hand in hand with the increase in moisture up to optimum, beyond which it decreases.

The eighteen-day period again shows that the optimum physical moisture corresponds closely to the optimum moisture conditions for the fungi, except in the case of the cottonseed meal, where one-half optimum gave better results.

The fact that moisture variation has not seemed to alter appreciably the behavior of the organisms at different periods, permits us to see at once the effect of time of incubation upon ammonia accumulation.

With the *Mucor* the increase from the sixth to the twelfth day was marked, while no appreciable advantage was gained by incubating eighteen days. However, with the *Penicillium*, in eighteen days there was still a large increase over that obtained at twelve days. But if we turn to Table III we find that with cottonseed meal less ammonia was noted at optimum than at one-half optimum, a point which might indicate that some other

process had started, thus consuming the ammonia where there was sufficient moisture. This fact in itself would tend to warrant the choice of a twelve-day period. One can easily see how the length of incubation might alter the relationship between organisms in ammonification studies.

TABLE III.  
AMMONIFICATION BY MUCOR PLUMBEUS AND PENICILLIUM SP. IN 18 DAYS.

Moisture	Organic comp.	Check		Mucor plumbeus			Penicillium sp.		
		Amm'ia N found		Ammonia N found			Ammonia N found		
		mg. N	Ave'ge	mg. N	Ave'ge	Inc. over check	mg. N	Ave'ge	Inc. over check
¼ optimum	100 mg. N. in Dried Blood	1.9		4.40			20.4		
		1.8	1.85	4.20	4.30	2.45	21.8	21.10	19.25
		1.9		13.15			31.1		
½ "	"	2.2	2.05	14.05	13.60	11.55	30.2	30.65	28.60
		1.8		21.90			36.3		
		1.8	1.80	22.90	22.40	20.60	35.7	36.00	34.20
1 "	"	1.8		21.00			32.7		
		1.6	1.70	21.70	21.35	19.65	30.3	31.50	29.80
		1.9		20.45			12.6		
¼ optimum	100 mg. N. in Cot's'd Meal	1.6	1.75	19.25	18.85	18.10	19.9	16.25	14.50
		1.9		24.50			19.2		
		2.1	2.00	23.70	24.10	22.10	19.9	19.55	17.55
½ "	"	2.1		22.00			17.4		
		1.8	1.95	23.50	22.75	20.80	16.9	17.15	15.20
		2.1		20.60			15.5		
1 "	"	lost	2.10	20.40	20.50	18.40	16.8	16.15	14.05

In the work of McLean and Wilson,<sup>2</sup> where fungi were compared as to their capacity to ammonify, a seven-day period was employed. Had a longer period been chosen, which would have given the slower ammonifiers time to develop, no doubt different results would have been obtained. The *Aspergillaceae* group was found in their work to be composed of weak ammonifiers, and the *Mucors* of strong ammonifiers. It is interesting to note and compare the results obtained in the above tables with those in the work referred to. In ammonia accumulation the *Mucor* led by a large margin the *Penicillium*, a member of the *Aspergillaceae* group, with both dried blood and cottonseed meal for the six-day period. However, in twelve days the *Penicillium* with dried blood surpassed the *Mucor*, and nearly equalled it with cottonseed meal, holding this relationship through the eighteenth day. It appears that with slow growing organisms a longer period of incubation is necessary to secure truly characteristic activity.

In an effort to determine whether or not the biological stage of the fungi bears any relationship to their capacity to accumulate ammonia, the preliminary experiments served as a guide to the following plan.

<sup>2</sup> McLean and Wilson, Ammonification Studies with Soil Fungi. N. J. Agr. Exp. Sta., Bulletin 270, 29 p., 1 pl.

## SERIES II.

## The Biological Stage of Fungi as Affecting Ammonification.

The organisms, soil, and organic matter were the same as noted above. One hundred grams of soil were placed in Erlenmeyer flasks of 250 c.c. capacity, thoroughly mixed with the organic matter in portions containing 155 mg. of nitrogen, and brought to optimum moisture conditions. These were then plugged and sterilized as before, after which proper inoculation was made. One cubic centimeter of the inoculum contained spores as follows: *Mucor*, about 1,000,000; *Penicillium*, 100,000-200,000; *Monilia*, 3,000,00-4,000,000. Incubation was effected at about 27° C. Daily determinations of ammonia were made in duplicate for each organism with both organic materials. The development of the organisms was watched daily as well.

TABLE IV.

## DAILY ACCUMULATION OF AMMONIA IN THE SOIL BY MUCOR PLUMBEUS.

Period of Incubat'n Days	Dried Blood				Cottonseed Meal			
	Ammonia Nitrogen found				Ammonia Nitrogen found			
	mg. N.	Average	Inc. over check	Daily gain	mg. N.	Average	Inc. over check	Daily gain
1	2.35				4.55			
	2.64	2.50	0.07	0.07	3.97	4.26	0.07	0.07
	3.53				8.82			
2	3.82	3.68	1.25	1.18	8.65	8.74	4.55	4.48
	4.41				12.05			
3	4.85	4.63	2.20	.95	10.44	11.25	7.06	2.51
	5.00				14.70			
4	5.12	5.06	2.63	.43	13.26	13.98	9.79	2.73
	6.47				31.31			
5	6.47	6.47	4.04	1.41	26.75	29.03	24.84	15.05
	6.76				34.10			
6	7.20	6.98	4.55	.51	31.05	32.58	28.39	3.55
	8.06				38.96			
7	8.23	8.15	5.72	1.17	37.53	38.30	34.11	5.72
	8.23				41.31			
8	8.53	8.38	5.95	.23	39.10	40.21	36.02	1.91
	9.56				39.84			
9	12.64	11.10	8.67	2.72	40.87	40.36	36.17	.13
	10.58				41.60			
10	11.17	10.88	8.45	-0.22	40.86	41.23	37.04	.87
	11.32				42.92			
11	10.88	11.10	8.67	0.22	40.72	41.82	37.63	.59
	11.61				42.78			
12	11.73	11.17	8.74	0.07	45.42	44.10	39.91	2.28
	2.44				4.26			
Check	2.42	2.43			4.12	4.19		

In the column of daily gains of ammonia by *Mucor plumbeus*, one sees that the strongest activity occurred about every other day; and from actual observation of the growth of the organism, this seemed to coincide with the times immediately following the periods of active spore formation.

TABLE V.

DAILY ACCUMULATION OF AMMONIA IN THE SOIL BY *MONILIA SITOPHILA*.

Period of Incubat'n Days	Dried Blood				Cottonseed Meal			
	Ammonia Nitrogen found				Ammonia Nitrogen found			
	mg. N.	Average	Inc. over check	Daily gain	mg. N.	Average	Inc. over check	Daily gain
1	2.94				4.85			
	3.09	3.02	0.59	0.59	5.88	5.37	1.18	1.18
	4.50				33.96			
2	4.70	4.60	2.17	1.58	36.30	35.13	30.94	29.75
	13.38				44.10			
3	13.52	13.45	11.02	8.85	43.66	43.88	39.69	8.75
	20.00				50.86			
4	20.58	20.29	17.86	6.84	51.16	51.01	46.82	7.13
	23.08				51.30			
5	23.52	23.30	20.87	3.01	52.77	52.04	47.85	1.03
	26.17				53.65			
6	26.31	26.24	23.81	2.94	53.94	53.80	49.61	1.76
	27.05				56.30			
7	29.20	28.13	25.70	1.89	53.80	55.05	50.86	1.25
	32.78				57.18			
8	32.49	32.64	30.21	4.51	55.86	56.52	52.33	1.47
	34.69				57.92			
9	34.25	34.47	32.04	1.83	58.95	58.44	54.25	1.92
	39.39				59.68			
10	lost	39.39	36.96	4.92	61.25	60.47	56.28	2.03
	39.54				59.53			
11	40.13	39.84	37.41	.45	60.86	60.20	56.01	-0.27
	41.01				60.71			
12	41.90	41.46	39.03	1.64	59.09	59.90	55.71	-0.30
	2.44				4.26			
Check	2.42	2.43			4.12	4.19		

*Monilia sitophila*, known to be a very strong ammonifier, showed in this series the accumulation of large quantities of ammonia even in the first days of incubation. With dried blood, about half of the total amount registered for twelve days was accumulated in four days, while with cottonseed meal about 80 per cent was accumulated in the same length of time. Or, in other words, at least 15 per cent of the total nitrogen in dried blood and 30 per cent of that in cottonseed meal, had been ammonified in four days. Spores were abundant here in the early stages of growth, particularly about the second and third days, the greater number occurring earlier in the cottonseed meal cultures. The correlation between sporulation and ammonia accumulation seems again to hold true.

Turning to Table VI, one finds that very little ammonia accumulated within the first six days by *Penicillium sp.*, and only on the seventh day was a marked increase in ammonia noticed. Up until the sixth and seventh days there was no notable sporulation, but principally development of mycelium. Extensive sporulation was observed beginning at

this period and about every two or three days thereafter. The figures in this table after the six-day period show a similar variation in the amount of ammonia accumulated.

TABLE VI.

DAILY ACCUMULATION OF AMMONIA IN THE SOIL BY *PENICILLIUM* SP.

Period of Incubat'n Days	Dried Blood				Cottonseed Meal			
	Ammonia Nitrogen found				Ammonia Nitrogen found			
	mg. N.	Average	Inc. over check	Daily gain	mg. N.	Average	Inc. over check	Daily gain
1	2.77				4.23			
	2.69	2.73	0.33	0.33	4.07	4.15	—0.04	—0.04
	2.36				4.06			
2	2.48	2.42	0.02	—0.31	4.06	4.06	—0.13	—0.09
	3.20				4.50			
3	2.91	3.06	0.66	0.64	4.22	4.36	0.17	0.30
	4.05				4.89			
4	3.72	3.89	1.49	0.83	5.12	5.01	0.82	0.65
	4.66				6.55			
5	4.95	4.82	2.42	0.93	5.96	6.26	2.07	1.25
	4.80				7.72			
6	5.39	5.10	2.70	0.28	7.72	7.72	3.53	1.46
	7.94				15.73			
7	6.76	7.35	4.95	2.25	16.61	16.17	11.98	8.45
	8.08				18.41			
8	9.42	8.75	6.35	1.40	18.86	18.64	14.45	2.47
	10.88				25.73			
9	9.26	10.07	7.67	1.32	26.90	26.32	22.13	7.68
	11.83				26.46			
10	12.20	12.02	9.62	1.95	27.56	27.01	22.82	0.69
	15.88				36.82			
11	16.76	16.32	13.92	4.30	34.54	35.68	31.49	8.67
	15.88				37.90			
12	17.40	16.64	14.24	.32	38.80	38.35	34.16	2.67
	19.55				41.60			
13	19.25	19.40	17.00	2.76	41.44	41.52	37.33	3.17
	22.05				43.12			
14	23.50	22.78	20.38	3.38	46.60	44.86	40.67	3.34
	27.20				48.95			
15	28.66	27.93	25.53	5.15	47.33	48.14	43.95	3.28
	2.40				4.26			
Check	2.40	2.40			4.12	4.19		

The curves give a graphical expression to the foregoing statements and show to some extent the behavior of the individual organisms accentuating the variability in daily gains of ammonia. Attention is called to the fact that in general there is a period of low activity in the beginning, followed usually by a period of maximum activity, and later by one of lesser and varying activity. The intensity of the period of maximum activity has its reaction in the subsequent growth of the organism, as is to be expected, when there is a point of very large ammonia accumulation. The later development is more uniform, but where there is no such point of extreme activity the periods of extensive ammonification alter-



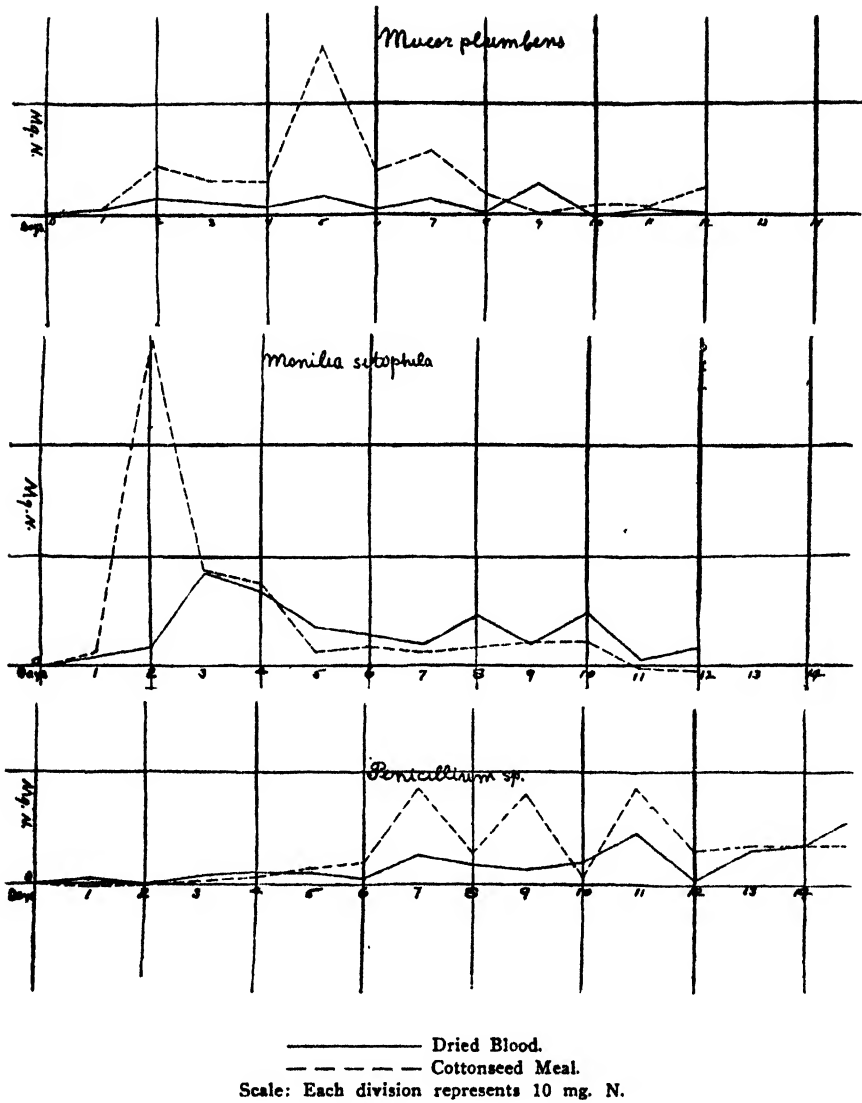


Figure 1.—Daily Ammonia Accumulation by (1) *Mucor plumbeus*, (2) *Monilia sitophila*, and (3) *Penicillium sp.*

nate with comparatively inactive ones. Each organism seems to have its own peculiarities in this respect. The application becomes of importance in considering data secured with different fungi. For instance, two or more organisms compared may give on one day a certain relationship to each other, while on another day this relationship may not exist, depending upon the respective sporulation periods.

It must not be understood, however, that ammonia accumulation depends upon sporulation; rather, it seems to follow it closely. The maximum gain in ammonia coincides with the germination of the spores and the subsequent development of the mycelium; while the minimum gain occurs when the organism prepares itself to produce new spores. While spore production may be a more or less continuous process, there are, however, well defined periods when it is the predominant activity of the organism.

A good analogy is found in the growth of legumes like the clovers. Nitrogen is fixed during the period of active growth of the plant, the fixation ceasing almost entirely when seed formation begins. In a like manner most of the ammonia seems to be accumulated when the spores of the fungus germinate to form new mycelium, this process being appreciably hindered at the time at which the organism forms its sporophores immediately preceding the complete development of the corresponding spores.

The three fungi studied represent three very important groups of soil organisms, and their behavior should therefore be indicative of the activities of those groups of which they are members. *Monilia sitophila* represents the Moniliaceae, which are strong ammonifiers, accumulating most of their ammonia in a very short period of time. This is undoubtedly due to their early and extensive spore formation. In contrast to the Moniliaceae, the Aspergillaceae, of which the *Penicillium sp.* is a member, are weak ammonifiers in short incubation periods; but if the period of incubation is sufficiently long, large quantities of ammonia will be accumulated. *Mucor plumbeus*, representing a third important group of soil organisms, places itself intermediate between the two above.

Taken collectively, the experiments show that there is an important connection between the biological stage of a fungus and its capacity to decompose organic matter as indicated by ammonia accumulation.

#### SUMMARY.

The results of these experiments indicate that:

1. Optimum moisture conditions for ammonia accumulation by fungi lie near the physical optimum.
2. The proper incubation period depends entirely upon the organism.
3. A twelve-day incubation period is preferable to a shorter one for practical work.

4. A correlation exists between the biological stage of the organism and the periods of ammonia accumulation; the largest amount seems to accompany the periods of spore germination and the smallest amount the time preparatory to actual spore formation.

5. *Monilia sitophila* shows the largest ammonia accumulation within the first three or four days; *Penicillium sp.*, between ten and fifteen days; and *Mucor plumbeus*, between six and ten days. These periods correspond to those of active spore formation for the respective organisms.

# A PRELIMINARY STATEMENT ON THE PRESENT STATUS OF THE HUMUS NITROGEN PROBLEM IN ARID SOILS.<sup>1</sup>

BY CHAS. B. LIPMAN,

*Professor of Soil Chemistry and Bacteriology, University of California.*

On the basis of analyses of soils for humus and humus nitrogen carried out by the late Professor Hilgard, his associate Professor Loughridge, and their assistants prior to 1912, the two investigators named came to the conclusion that soils of the arid region, while containing less humus than soils of the humid region, also contain a much higher percentage of nitrogen in the humus. This was taken as an indication of a tendency to equalization of the total nitrifiable nitrogen content in soils of the two regions. This view is emphasized in Hilgard's celebrated book entitled "Soils," published in 1907, and is widely quoted in other works on the same subject. Peculiarly enough, only the latter part of Hilgard's view with respect to the tendency toward equalization of the available nitrogen supply in soils of the two regions has been called in question, the facts supporting the first part being assumed to be correct. The criticism referred to was made by Stewart (4) in connection with his discussion of Headden's views on the origin of the "nitre" spots and Hilgard's views on the intensity of nitrification in arid soils. After pointing out on the basis of his studies on the nitrate content of Utah soils that there seems to be no evidence there of intense nitrification, Stewart suggests besides that Hilgard's explanation to the effect that the lack of "nitrogen hungriness" in California and other arid soils is to be accounted for as above pointed out is untenable and is "open to mathematical objections." Just what the "mathematical objections" are the writer is unable to see. It appears that Hilgard reasoned logically and correctly enough that if soils of the arid region contained one-fourth as much humus as soils of the humid region but that the humus in the former was four times as rich in nitrogen as that of the latter that the total nitrogen of the humus portion of the soil organic matter should be the same in both cases. This, it appears to the writer, is a simple arithmetical calculation the correctness of which cannot be questioned, and stands true regardless of the disparity in the nitrogen content of the unhumified organic matter of the

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two classes of soils. On what he regarded as the correct facts with reference to the nitrogen content of the humus of soils of the two regions, supplemented by the experimental results (1) obtained by him which indicated that unhumified organic matter supported little or no nitrification, Hilgard evidently felt justified in considering that for immediate purposes at least, arid soils were as little likely to be nitrogen hungry as humid soils. Even in the more recent critical work on the humus and humus nitrogen of the soil columns published by Loughridge (3), the author, while not making any claims to the existence of a higher nitrogen content in the humus of arid than in that of humid soils, appears to be still of that general opinion.

Taking all of these observations together, the writer cannot help but feel that with the exception of his ideas on the intensity of nitrification in arid soils which Stewart showed to be questionable, and on which the present writer and his associates soon hope to furnish more evidence of a direct nature, Hilgard's reasoning could be considered correct, but his facts were not correct. The latter is evidenced by the data submitted by Loughridge in the paper above cited, and by those in the writer's possession. In the first place, the humus of arid soils, considered by and large, is actually no richer in nitrogen than that of humid soils; and in the second place, Hilgard's single experiment to prove the superior nitrifiability of humified as against unhumified organic nitrogen is subject to serious criticism in the light of our present-day knowledge of the subject of nitrification.

In connection with some studies (2) made by D. D. Waynick and the writer, on the influence of climate on soil, a number of humus determinations were carried out. These determinations were made by the Grandeau method as modified by Hilgard, using at first only dilute ammonia. When, however, we attempted to determine the nitrogen in the ammonia extract, we found such large quantities of nitrogen in the humus that the figures were not used in connection with the investigation mentioned. It should be added that such high nitrogen figures were obtained after the humus solution had been boiled with magnesia for a period of four hours prior to digestion for the total nitrogen determination. Thinking, therefore, that we might obtain more satisfactory results by employing the recommendation of Hilgard for the use of a dilute solution of one of the fixed alkalies in the humus extraction, for purposes of the nitrogen determination, we used a 3 per cent solution of NaOH for the purpose, and obtained a totally different set of results. This occurred despite the fact that the humus extracts as obtained by the two methods looked about the same and yielded similar volumes of solution of similar color intensity. The figures for the humus and nitrogen determinations on the series of soils above discussed are given in Table I. The values for nitrogen in the humus are given as obtained by both methods.

TABLE I.

HUMUS AND HUMUS NITROGEN IN SURFACE SOILS FROM SOIL EXCHANGE PLOTS

	NH <sub>4</sub> OH Extraction		NaOH Extraction	Modified Gunning Method
	% Humus	% N in Humus	% N in Humus	Total % N in soil
California soil undisturbed .....	1.02	13.72	5.44	.096
California soil disturbed at California.....	1.00	14.70	4.13	.096
Maryland soil in California .....	1.22	14.91	4.30	.121
Kansas soil in California .....	1.05	11.61	5.00	.128
Maryland soil in Maryland .....	1.19	17.64	5.00	.099
Kansas soil in Maryland .....	.97	12.26	6.44	.120
California soil in Maryland .....	1.28	14.84	5.51	.089
California soil in Kansas .....	1.06	17.92	4.62	.092
Kansas soil in Kansas ..	1.15	12.78	5.18	.141
Maryland soil in Kansas .....	1.16	18.70	3.92	.110

In studying the data in Table I we see at once that the figures for nitrogen in the humus, in the case of the ammonia extract of the soils in question must be erroneous, since they indicate, in all but three cases, a larger amount of humus nitrogen than there is of total nitrogen in the soil as determined by the Gunning method. In the second place, it is striking to note the great discrepancy between the nitrogen content of the two humus extracts, the nitrogen of the NaOH extract averaging roughly only one-third as high as that of the ammonia extract. Moreover, the discrepancy is not a regular one which allows of the correction of error by a common factor, but the high value in one extract may become the low value in the other, and vice versa. In the group of soils under discussion, it is interesting to note what may be only accidental, that even the humus content does not differ notably as between the humid and the arid soils. They certainly give little support to the idea that the nitrogen content of arid soils is greater than that of humid soils. If anything, the figures support the contrary idea.

If, perchance, the soils above selected should be objected to on the ground that the arid soil of the group, namely the Davis soil, is an alluvial soil which is not truly arid in character, the following considerations will serve to reply to such objections. In an investigation by P. S. Burgess and the writer on the effects of irrigation on the physical, chemical and bacteriological characteristics of an Imperial Valley soil, which has been under way for more than three years, the humus and humus nitrogen determinations have been carried out among many others. The Imperial soil is of course a strictly arid soil, since the normal rainfall in that region is less than 2 inches a year. The soil has always been collected in a five-foot column, one sample as an average of every foot in depth for five feet being taken. Six semi-annual samplings of this kind have been made. At the first sampling we thought it would be possible to use the ammonia extract for the determination of humus and humus nitrogen. Obtaining by

such means, as in the foregoing case, much higher values for nitrogen in the humus than those obtained by the Gunning method for total soil nitrogen, an NaOH extract was prepared as before and analyzed for nitrogen. The results of the determinations gave a similar contrast between the two methods to that shown in Table I, except that it was more emphatic. Table II, setting forth the comparison, follows:

TABLE II.  
HUMUS AND HUMUS NITROGEN IN FIVE-FOOT COLUMN OF IMPERIAL SOIL.

Imperial Soil	NH <sub>4</sub> OH Extraction		NaOH Extraction	Modified Gunning Method
	% Humus	% N in Humus	% N in Humus	Total % N
First Foot .....	.325	22.09	4.73	.016
Second Foot .....	.325	17.23	5.15	.014
Third Foot .....	.560	11.25	5.58	.015
Fourth Foot .....	.375	17.28	4.30	.014
Fifth Foot .....	.425	16.47	4.73	.018

We note in Table II again the great discrepancy between the nitrogen contents of the NH<sub>4</sub>OH and NaOH extracts amounting by averages to more than three times the quantity in the former as, in the latter. It will be remembered that this figure is similar to the one obtained by rough comparison in Table I. This circumstance may, however, be merely a coincidence. Again, the highest value for nitrogen in the humus by the NH<sub>4</sub>OH extract method corresponds to the figure which is next to the lowest in the NaOH extract series. In Table II further it will be noted as in the case of Table I, that the figures for nitrogen in the humus by the ammonia method are far higher than is possible considering the total nitrogen in the soil. But Table II shows the same to be true in the case of the third foot of the Imperial soil even by the NaOH method. This is probably due to an error in the humus determination and is the only real exception to the rule that the nitrogen in the humus by the NaOH method is seldom as high as, and usually considerably lower than, the total nitrogen in the soil.

Taking together the results given in Tables I and II, we are compelled to conclude that the method of determining humus nitrogen in the ammonia extract of soils is a seriously faulty one, no matter how much care is employed in boiling the extract with magnesia. The method is indeed so faulty as to deserve immediate rejection by all those who are at all concerned with the correct determination of nitrogen in humus. In the second place, if the results above given are considered in connection with the largest part of the humus nitrogen data furnished by Loughridge in the paper above cited, there can be no question that the prevalent belief in the high nitrogen content of the humus of arid soils is in error. The facts in hand do not justify any belief in the higher nitrogen content of the humus in either the arid or the humid group of soils over each other.

## GENERAL DISCUSSION.

Almost coeval with the earliest soil investigations of Hilgard and his associates and students at the University of California were their studies on soil humus and humus nitrogen. Some work was reported on that subject in nearly every report of Professor Hilgard until 1904. It is unfortunate that the precise method of humus extraction specifying the form of weak alkali used is not given in every case. By whatever method, however, such extraction was carried out, it appears to have led the investigators mentioned to the same conclusion, namely that the lack of humus in soils of the arid region was largely compensated for by the much larger quantity of nitrogen contained in it as against that of humid soils. As pointed out above, some serious error must have crept into these investigations. The writer is informed verbally by Professor Loughridge, who was associated with Professor Hilgard during the greater part of the latter's scientific career, that Professor Hilgard rejected, about two or three years prior to his death, the data published by him on the humus and humus nitrogen of soils in the California Experiment Station Report for 1892-3-4. It is to be presumed that this implied a rejection of all similar determinations theretofore made. A specific report is here mentioned because it gives analyses for humus and humus nitrogen of soils which were later examined in the same laboratory and shown to contain very much less humus nitrogen than that shown in the report. This was discovered by comparison of data in the report above mentioned with others given by Professor Loughridge in the paper above cited.

Any data therefore which were obtained in a determination of the nitrogen in the ammonia extract of soils are clearly shown above to be entirely unreliable. The reason for the low nitrogen obtained in the humus of humid soils by the same method is puzzling. It may, however, be explained by the following theoretical consideration. Soils from humid regions taken by and large, are admittedly possessed of greater absorbent internal surface, due either to a higher organic matter content, a larger clay content, or to both. For this reason, the humid soil being extracted on the filter would absorb more ammonia from the solution employed for extraction than the arid soil. Hence less ammonia would be absorbed by the colloidal humus solution in the filtrate, and therefore the error in the humus nitrogen, due to absorption of ammonia nitrogen as above proved, would be very much decreased. The opposite would of course be true in "organic matter poor" or "clay poor" arid soil. The great avidity with which soils will absorb ammonia has already been commented on and proved by many investigators, including Hilgard himself. The latter has discussed this subject in his book which is referred to above. This fact would therefore appear to lend further support to the theory above expressed.



On the other hand, the data which were obtained in the method of fixed alkali extraction of the humus and which still show high values for humus nitrogen, cannot be explained by the foregoing considerations. The writer can only say that the method of calculation, absorption of ammonia from the laboratory air, or some other error of that nature, must be responsible for the high nitrogen values obtained. In my knowledge, whether in working with the same soils with which Hilgard's former associates worked, or with others, there has never been an instance of a higher humus nitrogen percentage than 7 or 8 when the KOH or NaOH extraction method for humus was employed.

In view of all these facts and queries, it appears necessary to make a still further critical examination of the method in question, using the same soils as were heretofore employed by this experiment station and of which supplies are still extant. This we shall proceed to do at once, and the writer hopes to be able to report soon not only the results to be obtained, but also a critical discussion of the humus determination itself and its value, together with such review of past work and theories as may be pertinent in that regard. The statement here made is, however, felt to be urgently necessary in explanation of the fact that the pristine position of this division of the California Experiment Station, with respect to humus and humus nitrogen, is one to which we no longer adhere. As successor to Dr. Hilgard in the direction of the work in soil chemistry at the University of California, a position which the writer has the great honor of holding, he feels it incumbent upon him to make this public statement.<sup>1</sup>

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<sup>1</sup> Since the above statement has gone to press and proof received, the author has noted the appearance of a brief paper by F. J. Alway and E. S. Bishop (5) dealing in part with the same subject and in substance agreeing with his statements. Some points of disagreement do exist, however, which will be discussed in our later publication based on more complete experimental data which we hope may soon be ready for the press.

## PRELIMINARY EXPERIMENTS ON SOME EFFECTS OF LEACHING ON THE SOIL FLORA.<sup>1</sup>

By C. B. LIPMAN, *Professor of Soil Chemistry and Bacteriology*, and L.  
W. FOWLER, *Student, University of California*.

Investigations carried out in this laboratory (7) which will soon be reported in detail have demonstrated that marked changes in the physical and chemical condition of alkali soil result from the leaching thereof for the purpose of the removal of the salt. These changes, taken together with the renewed efforts now being made in the direction of reclamation of alkali land by tile draining and flooding, have suggested to the senior author the query as to the effect of such procedure on the soil flora as well as on the chemical and physical constitution of the treated soil. The writers, therefore, decided to carry out first some preliminary experiments on the subject dealing with some of the effects on the soil flora, to a degree which might be indicative of future procedure. The results of these preliminary experiments are so striking as to merit publication prior to the amplification of the work under field conditions.

The soils employed were a blow sand from a peach orchard near Oakley and a clay loam from the University Farm at Davis, Cal. Unleached soils and soils leached in the presence or absence of different salts were all tested for ammonification, nitrification, and nitrogen fixation. The quantities of salts used, in cases in which each salt was added separately to the soil at all, were NaCl .1 per cent,  $\text{Na}_2\text{SO}_4$  .25 per cent, and  $\text{Na}_2\text{CO}_3$  .05 per cent. For purposes of a combination of salts we employed the following, NaCl .10 per cent,  $\text{Na}_2\text{CO}_3$  .05 per cent, and  $\text{Na}_4\text{SO}_2$  .10 per cent. It will be noted that all of these quantities of alkali may be regarded as relatively small and such as might not interfere at all seriously with many crop plants through direct physiological effects. In carrying out the leaching, quantities of soil with and without salts were placed on filters in large funnels, and all of them, with the exception of the normal soil, which was used as a check, were leached with distilled water in quantities sufficient to remove the salts. The soil therefore which received no salt in all series, but was leached, was treated with just as much distilled water as the salt treated soils. The usual chemical methods were employed in testing for the presence or absence of salts in the leachings. After the leaching was complete, all the soils to be tested were spread out in thin layers to dry in the air of the laboratory. When dry they were ground and sifted for use in the following tests.

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## AMMONIFICATION.

Fifty-gram portions of every soil type and condition to be studied were placed in tumblers and mixed in the dry state, with 2 per cent blood in Series I and with 2 per cent cottonseed meal in Series II. Water was then added in the usual way to make an approach as nearly as possible to optimum moisture conditions, the soils were again thoroughly mixed, the tumblers covered with a Petri dish cover, and incubated for 15 days at 25° C. At the end of the incubation period the soil was transferred from the tumblers to copper distilling flasks, magnesia added, and the ammonia present distilled into standard acid, the excess of which was titrated as usual. The results showing the amounts of ammonia produced in every soil are given in Table I. The amounts of ammonia present in the original soil in every case have been subtracted from the amounts found by the analytical procedure just described.

TABLE I.  
SHOWING AMOUNTS OF AMMONIA, IN MILLIGRAMS, PRODUCED BY LEACHED  
AND UNLEACHED SOILS.

## SERIES I.—DRIED BLOOD.

Name of Soil	Unleached soil	Leached soil	.1% NaCl plus leaching	.25% Na <sub>2</sub> SO <sub>4</sub> plus leaching	.05% Na <sub>2</sub> CO <sub>3</sub> plus leaching	All salts as above plus leaching
Oakley .....	56.00	48.58	54.74	46.34	43.26	45.64
Davis .....	92.26	89.74	97.30	107.66	83.16	111.30

## SERIES II.—COTTONSEED MEAL.

Oakley .....	28.14	30.10	31.78	25.48	25.76	22.26
Davis .....	21.00	31.64	27.72	34.86	25.48	32.34

The data in Table I, while indicating undoubted effects resulting from some forms of leaching, clearly indicate that such effects are neither profound nor regular. This is particularly true of the Davis soil and the results obtained therewith. Thus leaching in its various forms depresses the ammonifying power of the Oakley soil for blood, but stimulates the ammonia producing power of the Davis soil for cottonseed meal. In all but two cases, in which minor depressions occur, the ammonifying power of the Davis soil is also stimulated so far as blood nitrogen is concerned. Unlike the Davis soil, however, with respect to cottonseed meal, the Oakley soil is in most cases depressed in ammonifying power by leaching, and in the two cases in which it appears to be stimulating, namely, those of leaching without salt, and with NaCl, the stimulation is not great and may not be outside the boundaries of error in ammonification work. The greatest depression in ammonification with the Oakley soils occurs in the case of leaching in the presence of Na<sub>2</sub>CO<sub>3</sub> with blood as the ammonifiable material and amounts to about 23 per cent of the total amount of

ammonia produced in the unleached check of normal soil. When cottonseed meal is employed the greatest depression in ammonifying power of the same soil occurs when leaching is carried out in the presence of a mixture of all the salts and amounts to about 21 per cent of the amount of ammonia produced by the unleached check soil.

In general, therefore, a study of the results given in Table I seems to indicate a definite yet relatively small average depression in ammonifying power of the Oakley soil induced by leaching, no matter which of the two forms of organic nitrogen is employed. On the other hand, the Davis soil appears to be stimulated in ammonifying power for both forms of nitrogen through the process of leaching, a depression occurring only twice in the case of the dried blood series. One of these depressions is very slight and the larger one of the two amounts to less than 10 per cent of the amount of ammonia produced by the unleached check Davis soil. The exact causes of the differences between the behavior of the two soils, which has just been noted, can be given only when further researches, on the composition of the leachings from the soil, have been completed. We may perhaps be permitted to surmise that such causes are directly traceable to the difference in the permeabilities to water of the two soils. The Oakley soil being much more permeable to water and containing less actual and "potential" colloidal material than the Davis soil, will allow of a more ready removal of more or less soluble salts, including the important bases set free by exchange of bases in the case of leaching in the presence of salts. This must result in a much more dilute and hence less congenial medium for bacterial development in the Oakley soil. On the other hand, the inferior permeability to water of the Davis soil permits it to retain larger quantities of the bases set free by exchange, and hence stimulation to ammonification may result from the presence of the latter. These are merely mentioned here as tentative suggestions toward the explanation of the results given in Table I. The writers make no claim to the entire adequacy of these suggestions in the premises, nor do they believe they are free from flaws, but pending attainment of further experimental evidence on the subject, they may be of interest to our readers.

#### NITRIFICATION.

In the nitrification tests 100-gram portions of the soil above described, instead of 50-gram portions, were employed. Dried blood, cottonseed meal, sulfate of ammonia, and the soil's own nitrogen, were employed. They were incorporated with the soil after the manner used in the ammonification experiments, the first and second being used at the rate of 1 per cent of the air dry soil, sulfate of ammonia at the rate of 2 per cent, and the soil nitrogen in the quantity and condition naturally existing in every soil. Owing to the exigencies of laboratory work at the time, the period

of incubation was reduced, contrary to our usual practice in nitrification work, from thirty to twenty-four days. The nitrate determinations were made by the phenoldisulphonic acid method. The very striking results obtained are set forth in Table II, which follows.

TABLE II.

EFFECT OF LEACHING IN THE PRESENCE AND IN THE ABSENCE OF SALTS ON NITRIFYING POWERS OF SOILS. RESULTS IN MILLIGRAMS OF NITRATE PRODUCED.

Forms of Nitrogen Used	Unleached Soil		Leached Soil		.1% NaCl + leaching		.25% Na <sub>2</sub> SO <sub>4</sub> + leaching		.05% Na <sub>2</sub> CO <sub>3</sub> + leaching		All salts as above + leaching	
	Oakley Soil	Davis Soil	Oakley Soil	Davis Soil	Oakley Soil	Davis Soil	Oakley Soil	Davis Soil	Oakley Soil	Davis Soil	Oakley Soil	Davis Soil
Soil Nitrogen .....	2.30	3.80	.30	1.40	.20	.28	.30	2.60	.90	.00	.55	.00
Dried Blood Nitrogen ...	.10	13.80	.00	.28	.00	.00	.00	.00	.00	.00	.00	.00
C. S. Meal Nitrogen .....	2.90	11.80	.35	2.20	.00	.00	.00	1.10	.00	.00	.00	.00
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> Nitrogen .....	.60	4.80	.00	1.20	.00	.20	.00	1.30	.00	.00	.00	.00

The results given in Table II are as unequivocal an answer to our query with respect to nitrification as we could possibly expect. The harmful effects of leaching on the nitrifying flora of both soil types are clearly manifest both in the presence and absence of salts, but are very much more marked in the former. In a word, we appear to have almost entirely deprived the two soils of their nitrifying power for at least fertilizer nitrogen by means of the leaching. We certainly have done so when salts were present except possibly in the case of Na<sub>2</sub>SO<sub>4</sub>. The most harmful effect of salts plus leaching is to be noted in the case in which all salts were added to the soil and leached. This is so in spite of the fact that the total salt concentration employed was no greater than in the case in which Na<sub>2</sub>SO<sub>4</sub> alone was employed. The salt next in order of damaging effect is probably the Na<sub>2</sub>CO<sub>3</sub>, and the third the NaCl. Sodium sulfate appears to be definitely much less harmful than the others. It will be further noted that while the two soils are totally different in nitrifying powers in the unleached condition, they are similarly affected by leaching in the presence of salts. It appears also that the form of nitrogen which is least affected by the prior leaching is the soil's own nitrogen, despite the fact that it was the only one which was subjected to the leaching process. Finally, it is of great interest to note that, while the salts were all removed from the soil before the latter was used, the actual removal of the salt has, in the case of every one, left behind a characteristic effect. Thus, as between the three single salts the order of harmfulness beginning with the most harmful should probably be Na<sub>2</sub>CO<sub>3</sub>, NaCl, Na<sub>2</sub>SO<sub>4</sub>. It is therefore not unlikely that we find herein further support for the theory advanced elsewhere by the senior author (5), that one of the most

serious phases of alkali injury is due to the effect of the salt on the soil rather than on the plant which is only indirectly affected. This seems to be supported by the order of injury induced by leaching out the different salts which is above referred to.

Not only are the nitrification data of striking interest and significance when considered alone, but also when compared with the ammonification data above discussed. Whereas ammonification is doubtfully, or at least not profoundly affected by the leaching process as carried out by us, nitrification is not only profoundly affected and largely inhibited, but it is brought to an absolute standstill in some cases and almost so in others. The senior author desires therefore again to call the reader's attention to his discussion (2) with reference to the great disparity in the physiological requirements of the two processes in soils, since space in this paper will not permit of further discussion of that subject. Numerous points other than those above mentioned are brought to our attention by the data in Table II, but we must refrain in this preliminary communication on the subject from giving them expression. They will receive full attention in future papers.

#### NON-SYMBIOTIC NITROGEN FIXATION.

Like the ammonification and nitrification tests above described, the nitrogen fixation tests were carried out in soil cultures. To 50 gm. of soil 1 gm. of mannite was added and thoroughly mixed therewith. The necessary water was added and the mixture again thoroughly stirred. The tumblers were then covered with Petri dish covers and incubated for three weeks under the same temperature conditions as the cultures in the other series. At the end of the incubation period, the cultures were dried at 100° C., ground and analyzed for nitrogen by the method (3) in use in this laboratory. The results showing the amounts of nitrogen fixed are given in Table III, which follows.

TABLE III.

SHOWING THE AMOUNTS OF NITROGEN FIXED, IN MILLIGRAMS, PER GRAM MANNITE USED IN THE VARIOUS CULTURES.

Name of Soil	Unleached soil	Leached soil	.1% NaCl plus leaching	.25% Na <sub>2</sub> SO <sub>4</sub> plus leaching	.05% Na <sub>2</sub> CO <sub>3</sub> plus leaching	All salts as above plus leaching
Oakley .....	11.20	11.20	7.70	7.00	8.40	7.00
Davis .....	19.25	14.35	7.00	11.90	1.40	3.15

It is very clear from the foregoing data that the effect of leaching is quite manifest on the nitrogen fixing powers of the soils tested. In the case of the Oakley soil leaching has markedly decreased the nitrogen fixing power, but only in the soils from which added salts had been

leached. In the case of the Davis soil, even leaching without previous salt addition induced a marked inhibition to nitrogen fixation. While the depression as stated was marked in both soils, it was, however, much more marked in the Davis than in the Oakley soil. In other words, leaching affected nitrogen fixation in both soils much as it did nitrification and not in the manner of its action on ammonification. To be sure, the injury is not, speaking in the absolute, anywhere as great to the soil's nitrogen fixing power as it is in the case of its nitrifying power.

From the theoretical standpoint one would be led to expect that both nitrification and nitrogen fixation would be similarly affected in the Davis soil and more profoundly so than in the Oakley soil. This is for the reason that the plentiful supply of colloids which are contained in the Davis soil would be diffused by leaching, and through their diffusion would seriously limit the air supply which is so essential to the two processes in question. No doubt, however, other effects induced by leaching operate toward the same end as the limitation of the air supply, but we shall defer discussion of them until more data have been gathered. It is interesting to note here, however, that  $\text{Na}_2\text{CO}_3$  alone, or with the other salts, appears to be the most injurious salt in the case of both nitrification and nitrogen fixation, even though only the residual effects of its erstwhile presence in the soil remain. This is particularly noteworthy here because it is in accord with findings (1, 4) by the senior author on the effect on the nitrifying and nitrogen fixing bacteria of  $\text{Na}_2\text{CO}_3$  as compared with the other salts when the latter are allowed to remain in the soil.

#### CONCLUDING REMARKS.

In addition to the studies on the effects of leaching on the ammonifying, nitrifying, and nitrogen fixing soil flora which are above reported, the senior author carried out an additional test with regard to the cellulose destroying bacteria. This consisted in placing in different Petri dishes in accordance with a method described in this journal (6), some unleached Davis soil, some leached Davis soil, and some leached Davis soil which had received prior to leaching .2 per cent KCl. These soils were properly moistened and discs of filter paper placed in contact with their surfaces. After two weeks' and even after four weeks incubation the leached soils showed no disposition to attack the filter paper. In the case of the unleached soil, however, after only two weeks most of the filter paper had disappeared.

In general, therefore, while it is still too early to draw any hard and fast conclusions from the experiments above briefly discussed, it seems quite certain that leaching affects the bacterial flora of soils profoundly. From the evidence adduced from our experiments, this is particularly so for the nitrifying, nitrogen fixing and cellulose destroying organisms. All of these processes appear to be wholly or almost wholly checked by

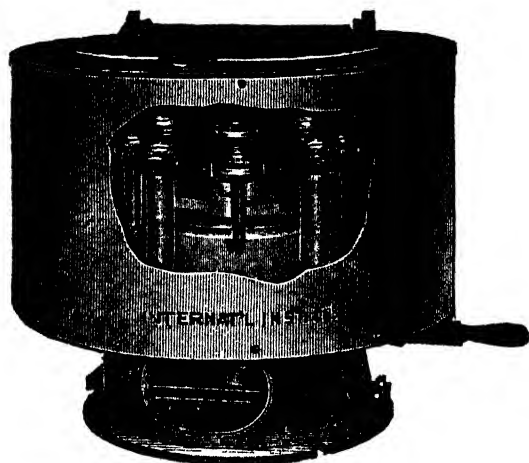
leaching, especially if salts are present prior to the execution of the latter process. It now remains to be seen if leached and underdrained alkali soils are injured as were the soils above studied, and if the injury done is or is not merely an ephemeral one which may entirely disappear in a few months under field conditions. The authors are now proceeding to a study of these questions in alkali soils in the field. Also they are inaugurating experiments on the effects of leaching alone, in the absence of salts, which should, in view of the results reported above, also yield highly interesting results. The latter will be particularly important from the practical standpoint because of their cogency in connection with all irrigation operations, and particularly with that of the practice of irrigation by flooding. Much damage has already been noted on relatively new soils where the last named system has been in vogue, and it is hoped that the experiments may lead to the discovery of the cause or causes of such damage.

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# SOIL SCIENCE

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## THE LOESS SOILS OF THE NEBRASKA PORTION OF THE TRANSITION REGION :

### III. POTASH, SODA, AND PHOSPHORIC ACID.<sup>1</sup>

By F. J. ALWAY, *Chief, Division of Soils, Agricultural Experiment Station, University of Minnesota*, AND R. M. ISHAM, *Chief Chemist, Research Department, Pittsburg Testing Laboratory*.<sup>2</sup>

#### INTRODUCTION.

In Nebraska the loess extends westward for about 300 miles from the eastern boundary on the Missouri River. Throughout this distance the temperature conditions are quite uniform, but there is a gradual decrease in the humidity of the climate, the normal annual precipitation, which exceeds 30 inches at the eastern boundary, steadily falling until it is less than 20 inches in the extreme western portion, while the rate of evaporation increases considerably. The climate of this region has been considered in detail in a previous paper (3).

The soil samples, upon which this article is based, were collected from 30 virgin prairie fields, 5 near each of six stations of the United States Weather Bureau shown in figure 1—Wauneta, McCook, Holdrege, Hastings, Lincoln, and Weeping Water. In each field, at intervals of 30 feet, ten borings were made to a depth of 6 feet and composite samples prepared of each foot-section, thus giving 6 samples from each field, the so-called "field samples." From these we prepared the "area samples," by mixing equal weights of the corresponding five "field samples." Thus each of the "area samples" is a composite from 50 individual borings. The details of the methods of sampling are given in the article referred to above.

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<sup>1</sup> Received for publication February 10, 1916.

<sup>2</sup> The work reported in this paper was carried out during the summers of 1911 and 1912 at the Nebraska Agricultural Experiment Station, where the authors were Chemist and Assistant in Chemistry, respectively. Mr. J. W. Tobiska assisted during the latter summer.

## TOTAL AMOUNTS PRESENT.

In the case of the "area samples" the total potash and soda were determined by the J. Lawrence Smith method, and the total phosphoric acid by digestion with hydrofluoric and nitric acids (12, p. 163). Also, the proportion of each of the three constituents dissolved by a five-day digestion with strong hydrochloric acid (7, p. 18) and the portion of the potash and the phosphoric acid soluble in a 1 per cent citric acid solution (5), were determined. In the case of the first-foot "field samples" from all thirty fields the total phosphoric acid was determined as above mentioned and the total potash by the modified J. Lawrence Smith method (11), which does not include the soda.

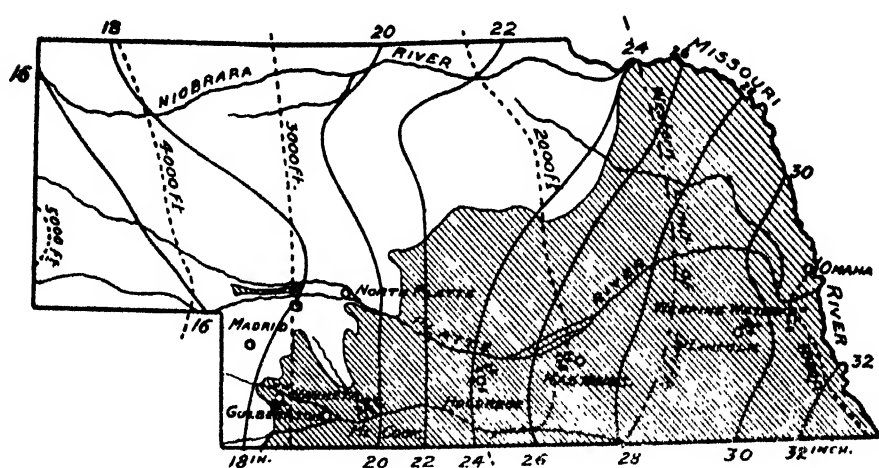


Fig. 1—Map of Nebraska showing distribution of the loess (shaded), annual precipitation and location of the fields sampled.

The total potash and soda in the area samples are reported in Table I. The distribution of potash throughout the extensive region is very uniform, whether the different depths of the same area, or the different areas are compared. While on the whole the potash content is somewhat higher in the deeper sections the variations are small and irregular. The two eastern areas show slightly lower, and the extreme western slightly higher, amounts than the intervening three. The distribution of the soda shows greater differences. In the western four areas it is quite uniform, both from the surface downward and from area to area, but in the eastern two the amount of soda is distinctly less than in the others, and also is greater in the lower than in the upper three feet.

The data reported in Table I were included in a previous article by one of the authors (1). Later, in checking over the analyses, it was found that an error had been made in introducing corrections for the soda and the potash contained in the reagents, the correction for the former having been deducted from the found percentage of the latter and vice versa. Accordingly the data in the article referred to are .07 or .08 per cent too low for potash and too high for soda. In the case of a few of the samples new determinations have been made.

TABLE I.

TOTAL POTASH AND SODA IN THE FOOT-SECTIONS FROM THE SIX AREAS.

POTASH ( $K_2O$ ).

Depth Foot	Wauwata %	McCook %	Holdrege %	Hastings %	Lincoln %	Wpg.Wtr. %	Average %
1	2.63	2.51	2.40	2.49	2.46	2.46	2.49
2	2.68	2.49	2.46	2.45	2.47	2.38	2.49
3	2.70	2.50	2.56	2.51	2.51	2.42	2.53
4	2.65	2.55	2.67	2.56	2.54	2.37	2.56
5	2.67	2.63	2.64	2.67	2.52	2.45	2.60
6	2.75	2.60	2.66	2.65	2.53	2.42	2.60
Average	2.68	2.55	2.56	2.55	2.50	2.42	2.54

SODA ( $Na_2O$ ).

1	1.41	1.50	1.50	1.48	0.96	1.05	1.32
2	1.43	1.49	1.38	1.36	0.91	0.99	1.27
3	1.34	1.40	1.40	1.36	1.06	1.04	1.27
4	1.42	1.36	1.44	1.59	1.14	1.29	1.37
5	1.45	1.51	1.57	1.47	1.21	1.27	1.41
6	1.48	1.50	1.49	1.54	1.18	1.37	1.43
Average	1.42	1.46	1.46	1.47	1.08	1.17	1.34

In the surface foot the potash varies only little from field to field in the same area (Table II), but is slightly higher in the fields near Wauwata than in those of the more easterly areas.

TABLE II.

TOTAL POTASH IN THE SURFACE FOOT OF THE DIFFERENT FIELDS OF EACH OF THE SIX AREAS.

Field No.	Wauwata %	McCook %	Holdrege %	Hastings %	Lincoln %	Wpg.Wtr. %
I	2.62	2.52	2.46	2.57	2.42	2.46
II	2.59	2.46	2.53	2.43	2.44	2.47
III	2.59	2.54	2.52	2.49	2.40	2.43
IV	2.59	2.53	2.48	2.51	2.45	2.43
V	2.60	2.56	2.47	2.61	2.44	2.43
Average	2.60	2.52	2.49	2.52	2.43	2.44

The total phosphoric acid, while showing a greater variation than the potash, is also quite evenly distributed (Table III). In the first foot and in the second it seems much the same throughout, but in the deeper sections it shows a higher content in the two eastern areas. In the surface foot-samples from the thirty fields (Table IV) it shows almost as great a uniformity as the potash except that it is somewhat lower in all the fields of the Hastings area. The data on the amounts in the first and second feet, compared with those in the lower levels of the Lincoln area, as reported in Table V, indicate that the subsoil in this area is uniformly richer in phosphoric acid than the surface soil.

TABLE III.

TOTAL PHOSPHORIC ACID IN THE FOOT-SECTIONS FROM THE SIX AREAS.

Depth Foot	Wauneta %	McCook %	Holdrege %	Hastings %	Lincoln %	Wpg.Wtr. %	Average %
1	.124	.135	.140	.107	.132	.126	.127
2	.129	.122	.113	.107	.139	.115	.121
3	.116	.115	.131	.116	.160	.125	.127
4	.151	.117	.151	.108	.166	.160	.142
5	.149	.128	.130	.135	.187	.182	.152
6	.137	.129	.108	.147	.171	.171	.144
Average	.134	.124	.129	.120	.159	.146	.135

TABLE IV.

TOTAL PHOSPHORIC ACID IN THE SURFACE FOOT OF THE DIFFERENT FIELDS OF EACH OF THE SIX AREAS.

Field No.	Wauneta %	McCook %	Holdrege %	Hastings %	Lincoln %	Wpg.Wtr. %
I	.139	.132	.139	.111	.130	.130
II	.131	.132	.139	.104	.146	.120
III	.125	.132	.139	.105	.140	.126
IV	.125	.126	.132	.105	.146	.126
V	.145	.139	.145	.119	.132	.132
Average	.133	.132	.139	.109	.139	.127

TABLE V.

TOTAL PHOSPHORIC ACID AT DIFFERENT DEPTHS IN THE FIVE FIELDS OF THE LINCOLN AREA.

Depth Foot	Field I %	Field II %	Field III %	Field IV %	Field V %	Average %
1	.130	.146	.140	.146	.132	.139
2	.146	.147	.146	.138	.117	.139
3 & 4	.168	.160	.161	.163	.171	.165
5 & 6	.184	.175	.165	.178	.160	.172

## HYDROCHLORIC ACID SOLUBLE PORTIONS.

Both potash and soda were determined by Hilgard's method of acid extraction, digesting with hydrochloric acid of 1.115 sp. gr. for 5 days over the steam bath (7, p. 18). In the same extract the phosphoric acid was determined, but it should be pointed out that this is not the method of analysis for phosphoric acid employed by Hilgard, who ignites the sample and then digests it with strong nitric acid for 2 days. The data reported on these hydrochloric acid soluble fractions (Table VI) are from single analyses, while for the total constituents we use the average of concordant duplicate determinations. In some cases the acid-soluble portion is clearly out of place, even exceeding the total. The exceptional values referred to, however, do not materially affect the results as a whole. In order to determine finer differences it would be necessary both to make duplicate determinations and to use a weight of soil greater than we employed, viz., 2 to 4 gm.

TABLE VI.

POTASH, SODA AND PHOSPHORIC ACID, IN FOOT-SECTIONS FROM THE SIX AREAS, DISSOLVED BY 5-DAY DIGESTION WITH HYDROCHLORIC ACID OF SP. GR. 1.115.

## POTASH.

Depth Foot	Wauneta %	McCook %	Holdrege %	Hastings %	Lincoln %	Wpg.Wtr. %	Average %
1	.96	1.15	1.13	1.15	1.09	1.25	1.12
2	1.17	1.23	1.35	1.42	1.14	1.42	1.29
3	1.06	1.27	1.33	1.46	1.16	1.43	1.28
4	1.14	1.22	1.36	1.36	1.26	1.37	1.28
5	1.16	1.21	1.32	1.38	1.29	1.38	1.29
6	1.16	1.22	1.32	1.35	1.27	1.37	1.28
Average	1.11	1.22	1.30	1.35	1.20	1.37	1.26

## SODA.

1	.32	.47	.32	.48	.39	.23	.37
2	.37	.41	.42	.45	.45	.26	.39
3	.43	.49	.50	.46	.43	.33	.44
4	.33	.45	.50	.54	.51	.29	.44
5	.53	.41	.45	.46	.48	.37	.45
6	.43	.38	.47	.42	.46	.33	.41
Average	.40	.43	.44	.47	.45	.30	.42

## PHOSPHORIC ACID.

1	.121	.121	.115	.104	.115	.105	.114
2	.119	.108	.105	.111	.119	.104	.111
3	.100	.110	.127	.118	.115	.111	.114
4	.133	.105	.137	.105	.145	.159	.131
5	.130	.105	.130	.133	.135	.159	.132
6	.124	.108	.092	.127	.140	.166	.126
Average	.121	.110	.118	.116	.128	.134	.121

The acid-soluble potash is lowest in the two western areas, reaching a minimum at Wauneta, where the total potash is highest, but where also there is the highest proportion of very fine sand. In each area the proportion soluble in acid is somewhat the lowest in the surface foot. The proportion of the total soda soluble in acid is lower, varying from 22 to 36 per cent, shows no distinct dependence upon the depth, and does not differ between the humid and the semi-arid areas. The proportion of the total phosphoric acid dissolved averages over 90 per cent, the lowest found being 82, and shows no dependence upon the depth.

#### COMPOSITION OF THE SOIL SEPARATES.

To determine whether the separates—clay, silt, etc.—from the humid eastern areas differ chemically from the corresponding ones from the western semi-arid portion, two subsoil composites were prepared and subjected to mechanical analysis. For the former we used a composite of equal weights of the third, fourth, fifth and sixth foot area samples from Lincoln, and for the latter a similar composite from Wauneta.

TABLE VII.  
COMPOSITION OF SOIL SEPARATES FROM A HUMID (A) AND A SEMI-ARID SUBSOIL (B).

	Mech'l Analyses		Potash		Soda		Phos. Acid	
	A %	B %	A %	B %	A %	B %	A %	B %
Coarse to fine sand (— to 0.1 mm.)	1.94	3.25	1.46	2.83	1.25	1.67	.....	0.057
Very fine sand (0.1 to 0.05 mm.)	5.02	54.98	2.25	2.65	1.81	1.94	0.096	0.051
Silt (0.05 to 0.005 mm.).....	56.83	22.41	2.56	2.64	1.73	1.40	0.096	0.057
Clay (0.005 to —).....	32.24	14.24	2.19	2.42	0.33	0.37	0.126	0.120
Material soluble in HCl.....	<sup>1</sup> 1.72	15.25	2.21	2.76	6.22	1.24	5.820	1.850
Original material .....	100.00	100.00	2.53	2.70	1.24	1.42	0.171	0.138
Portion of material dissolved by the 1% HCl .....	<sup>1</sup> 1.72	15.25	0.038	0.145	0.107	0.065	0.102	0.097

<sup>1</sup> By difference.

In order to disintegrate thoroughly the floccules in which the soil particles were cemented together by carbonates, the soil was washed on the filter with hydrochloric acid until the washings gave no test for lime or magnesia. It was then washed free of acid and separated into clay, silt, very fine sand and a coarser fraction, which included the fine, medium and coarse sands. In each case the medium sand constituted about one-fourth of this fraction, while coarse sand was practically absent. The leachings and wash waters were collected, evaporated, and analyzed for potash, soda and phosphoric acid. In the four soil separates the total potash, soda and phosphoric acid were determined by the methods described above. Table VII shows both the proportions of the different separates and the chemical composition of these.

In the humid subsoil the potash is somewhat higher in the silt, and very much lower in the coarsest fraction, than in either the clay or the very fine sand, while in the semi-arid sample it is similar in the silt and the very fine sand and higher than in the clay but lower than in the coarsest fraction. Thus the silt and the very fine sand from the western area are alike and only slightly richer in potash than the silt from the eastern area. These two fractions together have been found to constitute from 77 to 95 per cent<sup>1</sup> of the weight of each of the area composites, the silt predominating in all except the Wauneta samples, and the proportion of very fine sand being lowest in the eastern two areas. The coarsest fraction forms such a very small proportion of any of the loess samples that it may be ignored. In the three other groups of separates the variation in potash is comparatively slight, the clay, which contains the least, having about nine-tenths as much as the silt, which contains the most. The comparatively slight variation in the distribution of the potash over the region is in accord with the variations in mechanical composition, together with the found composition of the separates.

In both subsoils the soda was highest in the very fine sand and much the lowest in the clay, it being in the latter but slightly more than one-fourth as high as in the original material and less than one-fifth as high as in the very fine sand. These variations are extreme, compared with those found for the potash. Accordingly we should expect to find a greater variation in the distribution of soda, as is actually the case.

In the case of both subsoils the acid used to remove the carbonates dissolved out over half of the total phosphoric acid, but only a small part of the potash and soda. From the semi-arid subsoil there was dissolved about four times as much potash but only about half as much soda as from the humid subsoil. This in comparison with the data in Table VI indicates that the relative solubility of these constituents is not the same in a cold dilute, as in a hot, concentrated, mineral acid.

It should be pointed out that the composition of these separates—especially of the clay—is not to be expected to be similar to that of those obtained by the method commonly used in this country, namely, deflocculation by means of prolonged violent shaking with a very dilute ammonia solution, followed by repeated decantations of the “clay-water” and the evaporation of this along with the clay. Also, the proportions of the various separates are different from those found by the common method, the effect of the hydrochloric acid treatment being to increase the finest separate, the so-called “clay.”

<sup>1</sup> According to analyses by Mr. C. O. Rost, by the method described in Bureau of Soils Bulletin 84 (1912). These will be reported in the next paper in the series which will appear in *Soil Science*, Vol. I, No. 5, May, 1916.



## CITRIC ACID SOLUBLE PORTIONS.

The uniformity in the distribution of the total potash, and to a lesser degree also that of the total phosphoric acid, in these Nebraska loess soils is such that there seems to be little promise of results of interest in further such analyses. Almost the same may be said of the portions soluble in strong hydrochloric acid. The situation, however, seems entirely different when we consider only the portions soluble in weak solvents. Attention has already been called to the much greater solubility of the potash of the western subsoil, when cold 1 per cent hydrochloric acid is used. A study of the solubility in 1 per cent citric acid solution has given unexpected results. The data reported in Tables VIII, IX and X were secured by shaking 200 gm. of soil with 2,000 c.c. of 1 per cent citric acid solution at frequent intervals through 7 days (6, p. 159). The potash and the phosphoric acid were determined in 500 c.c. aliquot portions, corresponding to 50 gm. of soil. A difference of .001 per cent of  $P_2O_5$  or  $K_2O$  would be indicated by .008 gm. magnesium pyrophosphate or .0026 gm. potassium platinochloride, thus giving some actual significance to figures in the third decimal place. The duplicate digestions usually give a difference of less than .001 per cent, and none was accepted when this exceeded .002 per cent  $P_2O_5$  or  $K_2O$ . The irregular intervals which characterize shaking by hand seem to exert no distinct effect upon the amount dissolved, the duplicate digestions giving as concordant results when made at intervals of several weeks as when carried on side by side; also the results obtained by two analysts, the one working a year after the other, were strictly concordant. In the later work the tedious shaking by hand was replaced by the use of a machine. Seven days' shaking by hand gave the same results as five hours in the machine; eight hours with the latter gave no higher result than five, but three gave lower. The data reported in Table XIII and part of those in XI were obtained by using the five-hour agitation.

The citric acid soluble phosphoric acid and potash in the different foot sections are reported in Table VIII. The potash increases from east to west, being lowest in the two eastern areas and highest in the extreme western. In the former it decreases somewhat in passing from the first to the sixth foot, while in the latter it distinctly increases. The intervening three areas show an intermediate behavior. The distribution of the citric acid soluble potash agrees with neither that of the total amount nor with that of the part soluble in hot strong hydrochloric acid. The increase in the solubility from east to west, and, in the western areas, from the surface downward, accompanies an increase in the carbonate content which must serve to neutralize a portion of the acid and so to lessen its solvent action. Thus in the Wauneta area the carbon dioxide content of the six area-composites, ranged in order from the first to the

sixth foot, are: 0.09, 0.07, 0.59, 1.67, 1.78 and 1.68 per cent. The carbonate contained in 200 gm. of the sixth foot sample would be sufficient to neutralize just about half of the citric acid contained in 2,000 c.c. of the solution used.

TABLE VIII.  
POTASH AND PHOSPHORIC ACID SOLUBLE IN 1 PER CENT CITRIC ACID SOLUTION.

POTASH ( $K_2O$ ).

Depth Foot	Wauneta %	McCook %	Holdrege %	Hastings %	Lincoln %	Wpg.Wtr. %	Average %
1	.047	.044	.048	.044	.032	.036	.042
2	.058	.055	.049	.042	.025	.032	.043
3	.073	.062	.057	.046	.021	.025	.047
4	.083	.066	.061	.049	.020	.023	.050
5	.084	.068	.061	.051	.019	.016	.050
6	.083	.067	.067	.053	.020	.016	.051
Average	.071	.060	.058	.047	.023	.025	.047

PHOSPHORIC ACID ( $P_2O_5$ ).

1	.043	.040	.038	.021	.012	.010	.027
2	.045	.030	.044	.025	.016	.009	.028
3	.032	.029	.060	.047	.036	.025	.038
4	.029	.027	.050	.056	.046	.045	.042
5	.026	.026	.044	.057	.050	.057	.043
6	.026	.029	.041	.055	.055	.064	.045
Average	.033	.030	.046	.043	.036	.035	.037

To ascertain whether all the fields of the eastern areas are characterized by a greater amount of soluble potash in the upper layers of the soil, and those of the western areas by the reverse, determinations were made for the first and sixth foot samples from all the fields of the two extreme areas—Weeping Water and Wauneta (Table IX). In the former all the fields show an excess of potash in the first foot, it being about twice as great as in the sixth, while in the latter all show less in the first foot, in which it is, in general, about two-thirds as high as in the sixth.

TABLE IX.  
CITRIC ACID SOLUBLE POTASH IN THE DIFFERENT FIELDS OF THE OUTER AREAS.

Weeping Water				Wauneta		
Field	First Foot	Sixth Foot	Ratio <sup>1</sup>	First Foot	Sixth Foot	Ratio <sup>1</sup>
No.	%	%	%	%	%	%
I	.031	.017	55	.057	.080	140
II	.034	.015	44	.051	.082	161
III	.039	.016	41	.043	.091	212
IV	.029	.016	55	.052	.078	150
V	.035	.015	43	.050	.077	154
Average	.034	.016	48	.051	.082	163

<sup>1</sup> The percentage in the first foot = 100.

In contrast with the potash the citric acid soluble phosphoric acid, considering the amount in the whole six-foot layer, does not increase from east to west, but is similar in the extreme areas, with the maximum in the intermediate. In the surface two feet it increases from east to west, while in the fifth and sixth it decreases. In the more easterly areas it shows an increase from the surface downward, while in the most westerly area it decreases markedly. The data for the first and the sixth foot sections from the fields of the outer areas (Table X) indicate that this difference is an area characteristic.

TABLE X.  
CITRIC ACID SOLUBLE PHOSPHORIC ACID IN DIFFERENT FIELDS OF THE  
OUTER AREAS.

Weeping Water				Wauneta		
Field No.	First Foot %	Sixth Foot %	Ratio <sup>1</sup> %	First Foot %	Sixth Foot %	Ratio <sup>1</sup> %
I	.009	.049	544	.030	.026	87
II	.008	.050	625	.036	.031	87
III	.013	.053	408	.046	.028	61
IV	.013	....	...	.036	.028	77
V	.011	....	...	.034	.025	74
Average	.011	....	...	.036	.028	77

<sup>1</sup> The percentage in the first foot = 100

Thus we find that the citric acid soluble potash and phosphoric acid show very marked differences according to the depth of the soil layer, in the most easterly areas the former decreasing and the latter increasing, while in the most westerly area the conditions are reversed (fig. 2).

It was evident that the decrease in the amount of citric acid soluble phosphoric acid in the western areas might be due to the greater amount of carbonates in the lower sections. Hilgard (8, p. 339) has suggested that in using the citric acid extraction one make "allowance for such neutralization as may occur in the soil," but Hall (6, p. 160) considers that in attempting to establish the amount of immediately available plant food "no attempt should be made to add an extra amount of citric acid to combine with the carbonate; secondary solvent actions are set up both by the carbon dioxide evolved and by the calcium citrate formed; moreover, the real comparative basis of the method of analysis is destroyed."

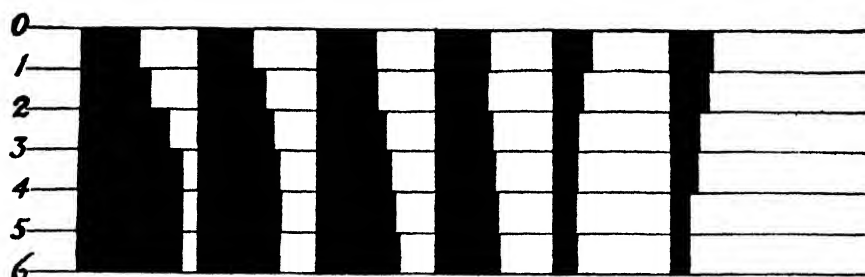
A bulk sample of loess subsoil taken at a depth of from 4 to 7 feet from the Experiment Station farm was subjected to eight successive extractions. After each extraction 1,000 c.c. of solution was removed for analysis and the remainder of the clear liquid decanted. In order to remove the citric acid solution remaining mixed with the soil the bottles were filled with water, shaken vigorously, and allowed to stand until the liquid became clear, when it was decanted, this operation being repeated

four or five times. Then there was added 20 gm. of citric acid and enough water to bring the liquid up to the mark at which it had first stood after adding the 2,000 c.c. citric acid solution to the 200 gm. of soil. The successive extractions removed gradually decreasing amounts

*Ft.*

*Wauneta. McCook. Holdrege. Hastings. Lincoln. W. Water.*

### POTASH



### PHOSPHORIC

### ACID

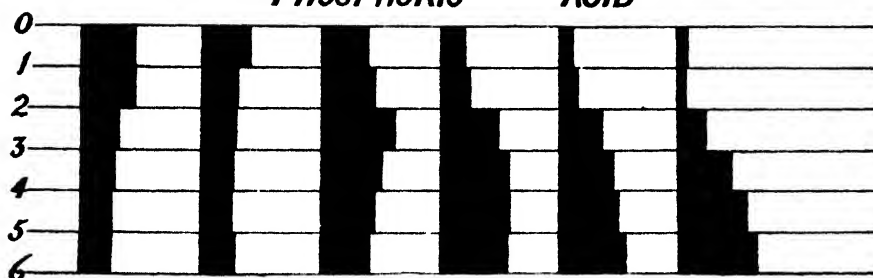


Fig. 2—Diagram showing relative amounts of citric acid soluble potash and phosphoric acid at different depths.

of phosphoric acid, this decreasing rapidly with the first three and slowly with the later ones, as shown in Table XI.

The depressing effect of the presence of calcium carbonate is shown by lines 5 and 6 in the above table. Some of the subsoil was in the one case mixed with 2 per cent and in the other with 6 per cent of calcium carbonate. Both of these were extracted at the same time as the untreated subsoil. Only .036 and .017 per cent, respectively, were dissolved from the first and second, while the last gave .061 per cent.

The first and the sixth foot samples from the fields of the outer areas, reported in Table X, were extracted a second time with citric acid, according to the method described above. As shown in Table XII, considerably less phosphoric acid was removed in the second extraction than in the first, except in the case of the calcareous subsoils of the Wauneta

area. In the latter area the two extractions together removed an average of 0.051 per cent from the first foot and 0.056 from the sixth, evidence that the decrease in solubility with increasing depth is to be attributed to the increase in the carbonate content and that such a decrease would not

TABLE XI.

AMOUNTS OF PHOSPHORIC ACID DISSOLVED BY 1 PER CENT CITRIC ACID SOLUTION, UNDER DIFFERENT CONDITIONS, FROM A SUBSOIL (4 TO 7 FEET) FROM THE LINCOLN AREA.

	Phosphoric Acid %
1. Shaken by hand 7 days .....	.061
2. Shaken in machine 3 hours .....	.056
3. Shaken in machine 5 hours .....	.060
4. Shaken in machine 8 hours .....	.061
5. After addition of 2% $\text{CaCO}_3$ , shaken by hand 7 days .....	.036
6. After addition of 6% $\text{CaCO}_3$ , shaken by hand 7 days .....	.017
7. Repeatedly extracted with fresh solution, the mixture being shaken 3 hours in the machine each time after the first extraction.	
1st extraction .....	.061
2nd extraction .....	.036
3rd extraction .....	.014
4th extraction .....	.008
5th extraction .....	.004
6th extraction .....	.004
7th extraction .....	.003
8th extraction .....	.002
Total removed by the 8 extractions .....	.132

have been found had we acted upon Hilgard's suggestion to allow for the neutralizing action of the carbonates.

TABLE XII.

RELATION OF THE AMOUNT OF PHOSPHORIC ACID REMOVED BY THE SECOND EXTRACTION WITH CITRIC ACID TO THAT REMOVED BY THE FIRST. WEEPING WATER.

Field No.	First Foot			Sixth Foot		
	1st Ext. %	2nd Ext. %	Ratio <sup>1</sup> %	1st Ext. %	2nd Ext. %	Ratio <sup>1</sup> %
I	.009	.008	90	.049	.036	73
II	.008	....	..	.050	.034	68
III	.013	.006	46	.053	.038	72
IV	.013	.006	46	....	....	..
V	.011	.006	55	....	....	..

## WAUNETA.

I	.030	.021	70	.026	.020	77
II	.036	.012	33	.031	.039	126
III	.046	.016	35	.028	.032	114
IV	.036	.011	31	.028	.030	107
V	.034	.017	50	.025	.019	76
Average	.036	.015	44	.028	.028	100

<sup>1</sup> The percentage in the first extraction = 100.

In the case of Fields I and III of the Weeping Water area, the only ones for which the data are complete, the first extraction removed 4.6 times as much phosphoric acid from the sixth as from the first foot, and the two extractions together 4.9 times as much. Thus the greater solubility of the phosphoric acid in the lower levels of the eastern area does not become any the less striking when a second extraction is employed.

It has been pointed out that in the case of the eastern areas the citric acid soluble phosphoric acid increases rapidly as we pass from the first to the sixth foot. To ascertain whether this increase is continued below the latter depth determinations were made of several deep samples which had been collected in connection with soil moisture studies (Table XIII). All, except Nos. 12 to 19, were from fields either on the Nebraska Experiment Station farm or not more than a mile distant, the loess on all varying from 15 to 25 feet in depth and overlying Kansan till. Nos. 12 to 18 are from a farm near Elgin, Nebraska, Nos. 17 and 18 being from an alfalfa field in which the loess deposit was found to have a thickness of 35 feet and in which the roots of the alfalfa plants had developed so freely to 30 feet that the subsoil to this depth had been practically exhausted of its available water (2, p. 118). No 19 is from a deep railway cut near Blair, Nebraska, where 25 feet of loess was removed over twenty years ago. The exposed loess subsoil has since been cropped. We do not know the depth of the loess at this place, but the deposit probably extends to at least 25 feet below the present surface, or 50 feet below the original. In general the high content of citric acid soluble phosphoric acid found in the sixth foot is continued into the deeper portions of the loess but without any marked increase. The low percentages in Nos. 11, 16, 17 and 18 seem directly connected with the presence of comparatively large amounts of carbonates.

Cameron (4, p. 77) estimates that the potash and phosphoric acid annually brought to the surface soil by capillary water is more than sufficient to replace the amounts that would be removed by "one ton per acre of dry crop containing 1 per cent potash and 0.6 per cent phosphoric acid." Where, as is the case with the soils of this study, no crop had been removed since the loess was laid down, we should expect to find a concentration of these nutrients in a readily soluble form in the surface soil. However, in the humid eastern areas we actually find a great exhaustion of phosphoric acid accompanying a distinct concentration of potash, while in the most arid areas of the west there is less readily soluble potash in the surface soil than in the lower levels, and the apparent concentration of phosphoric acid in the former is due to the neutralizing action of the carbonates in the latter.

In connection with the analytical data reported above it may be of interest to mention that, as the result of field observations and pot experiments on the loess soils of this region, we have concluded that alfalfa thrives almost as well upon the exposed loess subsoils of Eastern Nebraska as upon the surface soils. The former contain from 0.04 to 0.05 per cent nitrogen as compared with 0.20 to 0.30 in the latter. The "rawness" commonly attributed to humid subsoils must be entirely wanting, at least in so far as phosphoric acid and potash are concerned.

TABLE XIII.

CITRIC ACID SOLUBLE PHOSPHORIC ACID IN THE LOESS AT DEPTHS GREATER THAN SIX FEET.

Series No.	Location	Depth Foot	Composite from	Phosph'c Acid %
1	Lincoln .....	7-12	From excavation .....	0.073
2	Experiment Station Farm .....	7-10	From 2 borings .....	0.066
3	Near above farm .....	6-11	From 1 boring .....	0.067
4	Same boring as No. 3 .....	12-15	From 1 boring .....	0.044
5	Same field as No. 3 .....	6-11	From 1 boring .....	0.069
6	Same boring as No. 5 .....	1-15	From 1 boring .....	0.066
7	University Place .....	7-12	From 1 boring .....	0.067
8	Same boring as No. 7 .....	13-15	From 1 boring .....	0.075
9	Same field as No. 7 .....	7-12	From 1 boring .....	0.057
10	Same boring as No. 9 .....	13-15	From 1 boring .....	0.070
11	University Place .....	7-18	From 2 borings .....	0.038
12	Elgin .....	1-3	From 3 borings .....	0.015
13	Same boring as No. 12 .....	4-6	From 3 borings .....	0.044
14	Same boring as No. 12 .....	7-9	From 3 borings .....	0.040
15	Same farm as No. 12 .....	7-9	From 3 borings .....	0.047
16	Same boring as No. 15 .....	10-12	From 3 borings .....	0.024
17	Same farm as No. 12 .....	15-20	From new well .....	0.027
18	Same well as No. 17 .....	21	From bottom of same well	0.022
19	Blair .....	25	Exposed subsoil .....	0.057

Alfalfa probably makes a heavier draft upon the phosphoric acid than any other crop grown upon these soils. On one of the fields of this Experiment Station farm, which had been under cultivation a little over 40 years, alfalfa was grown from 1895 to 1907, all of it being removed as hay, giving an average yield of about 4 tons to the acre. No phosphate fertilizers, and little, if any, manure had been applied to the field. In 1912 composite samples were taken from each of the first six feet, 15 borings being used, and the citric acid soluble phosphoric acid was determined. The following percentages were found: 1st, 0.024; 2nd, 0.021; 3rd, 0.037; 4th, 0.041; 5th, 0.048; 6th, 0.075; average, 0.041. The first foot and the second show a considerably higher proportion than the average for the first and second foot of the five prairie fields of the Lincoln area, while for the other four feet the differences are not marked. In the alfalfa hay there was probably removed about 680 pounds of phosphoric acid and in the other crops about 450 pounds. The total, 1,130 pounds, would correspond to 0.037 per cent of the weight of an acre foot

of soil, assuming the latter to be 3,000,000 pounds, or more than the total amount of citric acid soluble phosphoric acid found in the first and second feet of the prairie fields. This affords a certain amount of support to the assumption that the plants have freely drawn for their supply upon the lower levels of the subsoil whenever their root distribution and the moisture conditions have permitted.

#### COMPARISON WITH CHERNOZEM AND ARID SOILS.

The Chernozem soils (10, p. 326) show a content of about 2.00 per cent total potash with small variations in both directions, while from 0.4 to 1.0 per cent is dissolved by 10 hours' digestion with 10 per cent hydrochloric acid on the water bath. The total soda is about 1.00 per cent, approximately half that of the potash, but a much smaller proportion of it is soluble in hydrochloric acid.

In the Nebraska loess soils the total potash and soda are somewhat higher, averaging 2.54 and 1.36 per cent, respectively, but bear about the same relation to one another. Digestion with hydrochloric acid of specific gravity 1.115 for 120 hours dissolved an average of 1.26 per cent potash and .42 per cent soda, the latter being here also the less soluble. The data on the acid soluble portions are not directly comparable, as the digestion of the Chernozem soils was made with a weaker acid and for a much shorter period.

The total phosphoric acid content of the Chernozem soils (10, p. 327) varies from .05 to occasionally more than .30 per cent, but usually the amounts lie between .15 and .30 per cent, the acid soluble portion constituting about four-fifths. This is appreciably higher than in the Nebraska loess soils, which show an average of .127 for the surface foot and of .135 for the first six feet, with a variation, in the case of very composite samples, between .104 to .184 per cent. In the Chernozem soils the phosphoric acid of the immediate surface (4 to 8 inches) is somewhat higher than in the underlying layers. The same has been found to be true for the Nebraska loess soils when different sections of the first foot are compared.<sup>1</sup>

The transition soils, while in general somewhat richer in total potash and slightly poorer in phosphoric acid, may on the whole be considered as very similar to the Russian Chernozem in potash, soda and phosphoric acid.

The data on the zeolithic (acid soluble) portions are directly comparable with those reported by Hilgard (9, p. 424), in a comparison of arid and humid soils. He finds the acid-soluble potash and soda to be much higher in arid than in humid soils, while the phosphoric acid in the one is similar to that of the other. The comparison is shown in Table XIV,

<sup>1</sup> Unpublished data of Alway, F. J., and Rost, C. O.



where the data for the surface foot samples from the Nebraska loess are reported. The soils from all the areas resemble arid soils, those from the most easterly as much as those from the western, semi-arid areas.

TABLE XIV.

COMPARISON OF THE TRANSITION SOILS WITH ARID AND WITH HUMID SOILS IN REGARD TO ACID-SOLUBLE POTASH, SODA AND PHOSPHORIC ACID.

	Potash %	Soda %	Phos. Acid %
Arid soils. Average of 313 soils (Hilgard) .....	.73	.26	.12
Humid soils. Average of 466 soils (Hilgard) .....	.22	.09	.11
Western two areas (from 10 fields) .....	1.05	.40	.12
Intermediate two areas (from 10 fields) .....	1.14	.40	.10
Eastern two areas (from 10 fields) .....	1.17	.31	.11

## SUMMARY.

The soils studied represent the first six foot-sections from five virgin prairie fields in each of six so-called "areas" in Nebraska, located between the Missouri River and the western limit of the loess, a distance of more than 300 miles, in which, while the temperature conditions, wind velocity and relative humidity are quite uniform, there is a great range in aridity, the annual precipitation decreasing from more than 30 inches in the east to less than 20 in the west, while the relative aridity exhibits a still greater range on account of the increase in the rate of evaporation which accompanies the decrease in precipitation.

The total potash is very uniform in distribution both from east to west and from the first to the sixth foot. While, on the whole, it is slightly lower in the eastern areas and in the higher levels, the variations are small and irregular. The proportion soluble in hot, strong hydrochloric acid seems largely dependent upon the amount of silt present, it being lowest in the most westerly area, in which, while the total potash is highest, the proportion of very fine sand also reaches its maximum.

The total soda shows somewhat more variation. In the western four areas it is quite uniformly distributed, both from area to area and from the surface downward, amounting, in general, to a little more than half as much as the total potash. In the two eastern areas it is distinctly lower; less is found in the upper than in the lower three feet, and in general it amounts to a little less than half as much as the total potash. The proportion of soda soluble in strong hydrochloric acid is lower than in the case of potash and is quite uniform.

The total phosphoric acid is still less evenly distributed. In the first two feet it seems much the same from east to west, while in the two eastern areas it is higher in amount in the lower than in the upper sec-

tions. Most of it is soluble in strong hydrochloric acid, neither location nor depth seeming to influence the proportion.

The loess consists chiefly of silt and very fine sand, with less than 5 per cent coarser than the latter. Determinations were made of the total potash, soda and phosphoric acid, as well as of the portions of these soluble in cold 1 per cent hydrochloric acid, in four separates—clay, silt, very fine sand, and coarser particles—from typical humid and semi-arid subsoils. In the very fine sand from the humid subsoil the amount of potash was found to be about the same as in the clay, but distinctly lower than in the silt. In the semi-arid subsoil it was similar in the silt and very fine sand, in both of which it was only very slightly higher than in the silt from the humid area, but was somewhat lower in the clay. In both subsoils the amount of soda was highest in the very fine sand and much the lowest in the clay. The dilute acid dissolved about four times as much potash, but only about half as much soda, from the semi-arid as from the humid subsoil, but the soluble portions of both form only a small proportion of the total amounts present. On the other hand, the dilute acid removed from both more than half the total phosphoric acid, the proportion dissolved being higher in the semi-arid subsoil. In the separates much more phosphoric acid was found in the clay than in the coarser fractions, the silt and the very fine sand, in which it was alike.

The most noteworthy differences were shown by treatment with citric acid solution. The potash soluble in this reagent was found to increase with the aridity; in the most humid areas it decreases from the surface downward, while in the least humid it increases, notwithstanding an accompanying increase in the carbonate content, which lessens the solvent action of the acid. In contrast with this, the citric acid soluble phosphoric acid was found not to increase with the aridity, when we consider the whole six-foot section; in the first two feet it increases, but in the lower four it decreases from east to west. In the most humid areas it increases rapidly from the surface to a depth of 6 feet, while in the most westerly areas it decreases. However, in the latter the difference is to be attributed to the increase in carbonate content, because when this is neutralized the sixth foot yields as much to the acid as does the first. The high content of citric acid soluble phosphoric acid is not confined to the lower portion of the six-foot sections, but continues to more than twice this depth. This high proportion of available phosphoric acid in the deep subsoil suggests a possible basis for a crop rotation in which deep-rooted legumes would provide both for the fixation of atmospheric nitrogen and for a transfer of phosphoric acid from the deep subsoil, through the medium of the plant parts, to the upper soil layers, in which the available portion is low and upon which the roots of annual and most perennial crop plants are dependent for their mineral nutrients.

In content of potash, soda and phosphoric acid the soils from all the areas resemble the Chernozem soils of Russia and the arid soils of California.

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# SOME FACTORS THAT INFLUENCE NITRATE FORMATION IN ACID SOILS.<sup>1</sup>

BY

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In earlier reports from this station, data were presented to show the formation and distribution of nitrates in cultivated soils (5, 6). This work was confined largely to a study of the distribution of nitrates in the soil and rate of nitrification throughout the growing period. No consideration was taken of the relation between soil reaction and the formation of nitrate nitrogen. Because of the large area of acid soil in the state of Wisconsin, studies of nitrification in certain types of acid soil have been undertaken.

The results of experiments made at Rothamsted show that nitrification is greatly diminished in soil rendered acid from manuring with ammonium chloride and sulphate (4). It appears that the number of nitrate-forming organisms is smaller in acid soils. Although it is reported that nitrification cannot take place in an acid medium, data from various sources show that this process is active in acid soil. For example, Temple of the Georgia Experiment Station reports that nitrification is active in a soil that requires more than 5,000 pounds of calcium carbonate per acre (10).

The following phases of nitrification were investigated: first, a study of the occurrence of nitrate-forming bacteria in acid soils and their relation to the organisms commonly found in neutral soils; second, a comparison of nitrification of organic and inorganic substances in acid and neutral soils; third, a comparison of the effect of calcium carbonate on ammonification and nitrification of organic substances.

Four types, representative of large areas of soil, were chosen. The control or neutral soil studied in connection with the acid soils was taken from the Station Farm. The soils were classified as follows: neutral Miami silt loam from Madison, acid Plainfield sand from Sparta, acid Colby silt loam from Marshfield, and acid peat from Wyeville. The reactions of the acid soils were as follows: Plainfield sand required 13.050

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pounds of calcium carbonate to neutralize one acre of soil 6 inches deep; Colby silt loam, 20,420 pounds, and Wyeville peat, 5,985 pounds<sup>1</sup>.

In order to prevent contamination, the soils were collected in large 8-gallon sterilized cans. After removing vegetation, the sample of soil was taken to a depth of 8 inches. The soil was then expressed to the Station Laboratory and prepared for experimental study.

Depending on the nature of the experiment, the soils were weighed into sterilized flasks, tumblers, or earthenware jars. With a few exceptions, the experiments were carried out at 60 per cent saturation and at a constant temperature of 25°C. Ammonia determinations were made by distilling with magnesium oxide in copper flasks; and nitrate determinations by the phenol-sulphonic acid method. In the case of soils high in organic matter or very rich in nitrates, the results from colorimetric analyses were compared with those obtained by the reduction method. All results are expressed as milligrams of nitrogen, per 100 grams of dry soil whether present as nitrate or ammonia.

#### OCCURRENCE OF NITRIFYING BACTERIA IN ACID AND NON-ACID SOILS.

*In Solution.*—The presence of nitrifying bacteria may be noted from the products formed in certain inorganic solutions. The Omelianski solutions used in this study are designed to favor the growth of these highly specialized organisms. For the nitrite bacteria, the nitrogen was added as ammonium sulphate; for the nitrate bacteria, it was added as sodium nitrite. The solutions were prepared, 25 c.c. portions in 300 c.c. Erlenmeyer flasks, and inoculated with 1-gm. samples of the various soils. The progress of the cultures was measured at regular intervals by qualitative tests. The following reagents were used: Nessler, Trommsdorf, and Diphenylamine. Table I contains the results of these tests.

Thirty days after inoculation, the Miami and Colby silt loams showed no ammonia, but a strong nitrite reaction. The conversion of nitrite to nitrate takes place even more rapidly. Twenty days after inoculation, all of the nitrite had disappeared and a strong reaction for nitrate was noted. In the case of acid sand and peat, oxidation was much slower. Enrichment cultures made by transferring a loop of the active cultures to new media gave similar results. Apparently the four types of soil contain the nitrifying organisms. When inoculated into a suitable culture medium, Miami or Colby silt loam soil nitrify much more rapidly than the acid sand or peat. The slowness of nitrification in the latter may be due to many factors, e.g., small number of organisms, decreased physiological efficiency. These factors will be discussed later.

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<sup>1</sup> Acidity determinations in sand and Colby soil were made according to the Truog Barium-hydroxide method (11); in peat according to the Veitch method.

*In Soil.*—The work was arranged to include not only investigations concerning the occurrence of the nitrifying organisms in acid soils, but also their activity when transferred to a sterilized soil of a different type. The bacteria from acid soils were transplanted to a non-acid soil and *vice versa*. In this way, an attempt was made to study the nitrifying flora of acid soils as compared with those of non-acid soils. The question naturally suggested itself: Are the nitrifying bacteria commonly found in acid soils more resistant to acidity than the same group of organisms from a non-acid soil? With this in mind, the following studies were made. Because of the similarity of these tests they are discussed in one group.

TABLE I.  
NITRIFICATION IN OMELIANSKI'S SOLUTION.

A.—NITRITE FORMATION

Time in days	Uninoculated		Soil							
			Miami		Sand		Colby		Peat	
	NH <sub>3</sub>	NO <sub>2</sub>	NH <sub>3</sub>	NO <sub>2</sub>	NH <sub>3</sub>	NO <sub>2</sub>	NH <sub>3</sub>	NO <sub>2</sub>	NH <sub>3</sub>	NO <sub>2</sub>
10	+	—	+	—	+	—	+	—	+	—
20	+	—	+	+	+	tr	—	+	+	tr
30	+	—	—	+	+	tr	—	+	+	tr

B.—NITRATE FORMATION.

Time in days	Uninoculated		Soil							
			Miami		Sand		Colby		Peat	
	NO <sub>2</sub>	NO <sub>3</sub>	NO <sub>2</sub>	NO <sub>3</sub>	NO <sub>2</sub>	NO <sub>3</sub>	NO <sub>2</sub>	NO <sub>3</sub>	NO <sub>2</sub>	NO <sub>3</sub>
10	+	—	+	—	+	—	+	—	+	—
20	+	—	—	+	+	tr	—	+	+	tr
30	+	—	—	+	+	tr	—	+	+	tr

— = no reaction.

+

= distinct reaction.

tr = trace.

Two-hundred-gram portions of the soil were weighed into 1-liter Erlenmeyer flasks, the moisture adjusted to 60 per cent saturation, and the soil heated in the autoclave for 1 hour at 15 pounds pressure. When cool, the sterile nitrogenous substances, ammonium sulphate and casein, were added in liquid form. The added nitrogen varied in the different experiments between 15 and 21 mg. per 100 gm. of soil. An exception to this is recorded in Table V. Here larger amounts were used. In each case the inoculum represented 5 gm. of soil. Each soil type was used as a medium and inoculated with the various soils. For example, Miami soil was used as a medium and inoculated with Miami, with Sparta sand, with Colby, and with peat. All determinations were made in duplicate and the averages given in the tables. The results for each soil are presented in Tables II to V.

*Miami soil.*—The data in Table II show, with one exception, that regardless of the source of inoculum, nitrogen from ammonium sulphate in neutral Miami soil nitrifies more rapidly than the same amount of nitrogen from casein. Within the time limit of this experiment, the acid Sparta sand and peat failed to nitrify. This agrees with the results of the test in solution.

It is apparent from the data that the activity of the nitrate-forming organisms in Miami soil is not very different from that of similar organisms in Colby soil. After 8 weeks the organisms from Colby oxidized the nitrogen of ammonium sulphate somewhat faster than those from Miami soil itself. Toward casein, the organisms from these two soils behaved alike. Evidently the nitrate-forming flora of acid Colby soil is not inferior to that of the neutral Miami silt loam. When inoculated into a neutral soil, their power to form nitrates is as great as that of the original flora.

TABLE II.  
NITRIFICATION IN STERILIZED MIAMI SILT LOAM.  
September 10—October 8—November 7.

Culture	Inocula	Treatment per 100 gm. of soil	Nitrate Nitrogen per 100 gm. of soil Average of duplicate flasks						
			Amt. in control	After		Increase		Nitrogen rec'd as nitrate	
				4 wks.	8 wks.	4 wks.	8 wks.	4 wks.	8 wks.
		Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	%	%
1	Miami	15 N from $(\text{NH}_4)_2\text{SO}_4$ ...	3.9	12.1	14.0	8.2	10.1	54.6	67.3
2	Sand	15 N from $(\text{NH}_4)_2\text{SO}_4$ ...	3.9	3.7	3.7	...	...	...	...
3	Colby	15 N from $(\text{NH}_4)_2\text{SO}_4$ ...	3.9	11.6	14.74	7.7	10.84	51.3	72.0
4	Peat	15 N from $(\text{NH}_4)_2\text{SO}_4$ ...	3.9	....	....	...	....	....	....
5	Miami	15 N from casein.....	3.9	12.5	11.4	8.6	7.5	57.3	50.0
6	Sand	15 N from casein.....	3.9	3.5	3.57	...	....	....	....
7	Colby	15 N from casein.....	3.9	10.8	11.36	6.9	7.4	43.3	49.7
8	Peat	15 N from casein.....	3.9	...	....	...	...	...	....

*Sparta sand.*—The apparent absence of nitrification in Sparta sand may be due to one or more factors. First, the proper organisms may not be present; second, the conditions necessary for nitrification may not be present. If the former statement is true, then sterilized Sparta sand inoculated with the proper organisms should nitrify. Accordingly an experiment was made with Sparta sand. Here acid sand was used as a medium and inoculated with soil suspension from all of the soil types.

Nitrate tests after 8 weeks did not show any increase beyond that of the uninoculated control. The results clearly indicate that sterilized Sparta sand is not suitable for a rapid oxidation of ammonia. The evidence shows that the absence of nitrification in Sparta sand is not due entirely to lack of proper organisms. This experiment was repeated using

0.5227 gm. of calcium carbonate per 100 gm. of soil, enough to neutralize all of the active soil acidity. The results should answer the question: Does calcium carbonate produce favorable conditions for nitrification in acid Sparta sand? The data in Table III give an answer to this question.

It is realized that the technique employed is far from perfect and that sterilization of soil will no doubt seriously affect the process of nitrification. Prior to this study, acidity tests were made in order to measure the effect of heat on reaction. No decided change in reaction was noted.

From the figures in Table III it will be seen that calcium carbonate only partially corrects the adverse conditions for nitrification in sterilized Sparta sand. When sterile Sparta sand was reinoculated with organisms from sand or peat, no increase in the formation of nitrates occurred. The bacteria from Miami and Colby silt loam soils gave a slight increase in nitrates. It is evident from the data, that Sparta acid sand is not well adapted for nitrification of ammonium sulphate or casein.

TABLE III.  
NITRIFICATION IN STERILIZED SAND PLUS CALCIUM CARBONATE.  
November 25—December 28.

Cul- ture	Inocu- la	Treatment per 100 gm. of soil	Nitrate nitrogen per 100 gm. dry soil			
			Amount in control	After 4 weeks	Increase	Nitrogen recovered as nitrate
		Mg.	Mg.	Mg.	Mg.	%
1	Miami	14.2 N from $(\text{NH}_4)_2\text{SO}_4$	1.2	3.28	2.08	14.6
2	Sand	14.2 N from $(\text{NH}_4)_2\text{SO}_4$	0.4	....	....	....
3	Colby	14.2 N from $(\text{NH}_4)_2\text{SO}_4$	0.6	1.80	1.20	8.45
4	Peat	14.2 N from $(\text{NH}_4)_2\text{SO}_4$	0.3	....	....	....
5	Miami	14.2 N from casein. .	1.2	.66	....	....
6	Sand	14.2 N from casein. .	0.4	..	..	....
7	Colby	14.2 N from casein. .	0.6	1.01	.41	2.8
8	Peat	14.2 N from casein. .	0.3	....	....	....

*Colby silt loam.*—In Table IV are given the quantities of nitrate produced by bacteria from the various soil types when used to inoculate Colby silt loam. A study of the effect of calcium carbonate on nitrate formation was included. In Group B enough calcium carbonate was added to neutralize all soil acidity.

The figures of Table IV show the nitrate content after 9 weeks. In the ammonium sulphate series without calcium carbonate, there was no increase in nitrates except where inoculated with Miami soil, while in the casein series there was a decided gain in nitrates when inoculated with Miami. It is clear from the data, that inoculations from neutral Miami soil into Colby soil medium caused a great gain in nitrate nitrogen. When Colby soil was used to inoculate Colby, no formation of nitrates was noted except in the limed series. Here, the organisms from Colby behaved much the same as those from Miami.



In spite of wide variations between duplicates, it seems safe to conclude that the nitrifying bacteria from acid soils when inoculated into acid soils are not any more efficient in forming nitrates than the nitrifying bacteria from a non-acid soil. In regard to the nature of the nitrifiable substance in acid soil, organic nitrogen is oxidized to nitrates more rapidly than inorganic nitrogen (2, 10).

TABLE IV.  
RATE OF NITRIFICATION IN STERILIZED COLBY SILT LOAM

November 25—January 30.  
GROUP A.—UNLIMED SERIES.

Culture	Inocula	Treatment per 100 gm. of soil	Nitrate nitrogen per 25 gm. dry soil			
			Amount in control	After 9 weeks	Increase	Nitrogen recovered as nitrate
		Mg.	Mg.	Mg.	Mg.	%
1	Miami	14.2 N from $(\text{NH}_4)_2\text{SO}_4$	2.92	4.05	1.13	7.9
2	Sand	14.2 N from $(\text{NH}_4)_2\text{SO}_4$	3.42	2.60		..
3	Colby	14.2 N from $(\text{NH}_4)_2\text{SO}_4$	3.40	2.97	..	....
4	Peat	14.2 N from $(\text{NH}_4)_2\text{SO}_4$	3.08	2.76	..	..
5	Miami	14.2 N from casein. . .	2.92	6.42	3.50	24.6
6	Sand	14.2 N from casein. . .	3.42	2.88	..	....
7	Colby	14.2 N from casein. . .	....	....	....	lost
8	Peat	14.2 N from casein. . .	3.08	2.74	....	....

GROUP B.—LIMED SERIES.

1	Miami	14.2 N from $(\text{NH}_4)_2\text{SO}_4$	10.57	17.42	6.85	48.2
2	Sand	14.2 N from $(\text{NH}_4)_2\text{SO}_4$	2.84	3.03	trace	....
3	Colby	14.2 N from $(\text{NH}_4)_2\text{SO}_4$	6.26	11.90	5.64	39.7
4	Peat	14.2 N from $(\text{NH}_4)_2\text{SO}_4$	3.40	2.69	....	....
5	Miami	14.2 N from casein. . .	10.57	15.87	5.30	37.3
6	Sand	14.2 N from casein. . .	2.84	3.07	.23	....
7	Colby	14.2 N from casein. . .	6.26	12.50	6.24	43.9
8	Peat	14.2 N from casein. . .	3.40	2.51	....	....

When the Sparta sand or peat was used as inoculum no increase in nitrates occurred. At the end of 5 weeks no gain in nitrates was noted in the ammonium sulphate series, except where inoculated with Miami soil. Here there was a slight oxidation of the ammonium salt, about 8 per cent of the added nitrogen. In the casein series, Miami inoculation, the formation of nitrates proceeded much more rapidly; more than three times as much nitrate nitrogen was found. Unfortunately the results of the Colby inoculation were lost.

From the data of Group B, it will be seen that carbonate of lime under the conditions of this experiment has greatly stimulated the nitrification of ammonium sulphate. The percentage of nitrate nitrogen was greater in acid soil inoculated with Miami than in acid soil inoculated with Colby. When inoculated with acid sand or peat, no nitrification occurred. Where

casein nitrogen was used and the base soil inoculated with Miami, calcium carbonate did not prove so beneficial. In Group A, no lime, 24.6 per cent of the casein nitrogen was recovered as nitrate; in Group B, limed, 37.3 per cent. The greatest increase in nitrates in the casein series was noted in the soil inoculated with Colby; the smallest, in the soil inoculated with Sparta sand.

TABLE V.  
RATE OF NITRIFICATION IN STERILIZED PEAT.

November 25—January 30.  
GROUP A.—UNLIMED SERIES.

Cult- ure	Inocu- la	Treatment per 25 gm. of soil	Nitrate nitrogen per 25 gm. dry soil			
			Amount in control	After 9 weeks	Increase	Nitrogen recovered as nitrate
		Mg.	Mg.	Mg.	Mg.	%
1	Miami	28.4 N from $(\text{NH}_4)_2\text{SO}_4$	trace	3 73	3 73	13.1
2	Sand	28.4 N from $(\text{NH}_4)_2\text{SO}_4$	trace	trace		....
3	Colby	28.4 N from $(\text{NH}_4)_2\text{SO}_4$	trace	trace	...	.
4	Peat	28.4 N from $(\text{NH}_4)_2\text{SO}_4$	trace	trace	..	....
5	Miami	28.4 N from casein....	trace	9 61	9 61	33.8
6	Sand	28.4 N from casein. ..	trace	trace	..	....
7	Colby	28.4 N from casein....	trace	7 81	7 81	27.5
8	Peat	28.4 N from casein....	trace	trace	...	....

GROUP B.—LIMED SERIES.

November 25—January 8.

Cult- ure	Inocu- la	Treatment per 25 gm. of soil	Nitrate nitrogen per 25 gm. dry soil			
			Amount in control	After 6 weeks	Increase	Nitrogen recovered as nitrate
		Mg.	Mg.	Mg.	Mg.	%
1	Miami	28.4 N from $(\text{NH}_4)_2\text{SO}_4$	4.16	14.5	10.4	36.7
2	Sand	28.4 N from $(\text{NH}_4)_2\text{SO}_4$	trace	trace	....	....
3	Colby	28.4 N from $(\text{NH}_4)_2\text{SO}_4$	2 00	13.88	11.88	41.8
4	Peat	28.4 N from $(\text{NH}_4)_2\text{SO}_4$	trace	trace	....	....
5	Miami	28.4 N from casein....	4.16	13.9	9.74	34.4
6	Sand	28.4 N from casein....	trace	trace	....	....
7	Colby	28.4 N from casein....	2.00	12.5	10.5	36.9
8	Peat	28.4 N from casein....	trace	trace	....	....

*Peat.*—In Table V are shown the results of a study of nitrate formation in peat. Apparently sterilized acid peat is fairly well suited for the growth of the nitrifying organisms. As regards the source of nitrogen, the organic compound casein nitrifies much more rapidly than ammonium sulphate. There was a slight oxidation of ammonium sulphate in the peat inoculated with Miami soil. In the presence of calcium carbonate, inorganic nitrogen from ammonium sulphate nitrifies somewhat more rapidly than organic nitrogen from casein.

The inoculated but unlimed peat nitrifies slowly. This is shown from the figures of Table V.

A review of the tests in sterilized soils shows that nitrification takes place to a marked degree in certain acid soils. The form of the nitrogen to be oxidized plays an important part. Organic nitrogen from casein will nitrify much more rapidly in acid soil than inorganic nitrogen from ammonium sulphate. In the presence of calcium carbonate the nitrifying flora from Miami neutral soil or Colby acid soil will oxidize a large percentage of the nitrogen from inorganic or organic compounds. In the absence of calcium carbonate, the oxidation of ammonium sulphate is greatest where Miami soil is used as an inoculum. At this time no satisfactory explanation can be offered to account for the peculiar action of the organisms from Colby soil. It is hoped that the results of experiments now in progress will reveal some of the important agencies that affect nitrification.

TABLE VI.  
RATE OF NITRIFICATION IN MIAMI SOIL.

January 16—January 19.

GROUP A.—FORMATION OF AMMONIA

Culture	Treatment per 100 gm. of soil	Ammonia nitrogen per 100 gm. dry soil		
		Average	Increase due to treatment	Nitrogen in casein recovered as ammonia
	Mg.	Mg.	Mg.	%
1	None .....	3.38	.....	.....
2	100 CaCO <sub>3</sub> .....	3.50	.12	.....
3	30 N from casein .....	12.84	9.46	31.53
4	30 N from casein plus 100 CaCO <sub>3</sub> ..	13.44	10.06	33.53

GROUP B.—FORMATION OF NITRATES.

November 24—December 28.

Culture	Treatment per 100 gm. of soil	Nitrate nitrogen per 100 gm. dry soil			
		At beg.	At end	Increase	Nitrogen recovered as nitrate
	Mg.	Mg.	Mg.	Mg.	%
1	None .....	2.5	3.15	.65	.....
2	100 CaCO <sub>3</sub> .....	2.5	3.15	.65	.....
3	14.2 N from (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	2.5	7.25	4.75	28.8
4	14.2 N from (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> plus 100CaCO <sub>3</sub> ..	2.5	9.70	7.20	52.1
5	14.2 N from casein .....	2.5	8.33	5.83	36.4
6	14.2 N from casein plus 100 CaCO <sub>3</sub> .....	2.5	8.95	5.80	36.2

## AMMONIFICATION AND NITRIFICATION IN NON-ACID AND ACID SOILS.

Here 200-gm. portions of the various soils, except where otherwise indicated, were weighed into glass tumblers. After treatment, these were covered loosely with petri dishes and allowed to stand for varying intervals. Since it was arranged to use organic nitrogen, it is important to know the rate at which the nitrogenous substance is converted into ammonia. A comparison of nitrate formation in soil to which ammonium sulphate and casein have been added is not fair unless the rate of ammonia formation is known. However, it is very unlikely that nitrification is retarded because of the rate of ammonia production. As a rule, the formation of ammonia from casein is very rapid.

TABLE VII.  
RATE OF NITRIFICATION IN SPARTA SAND.

January 17—February 20.

## GROUP A.—FORMATION OF AMMONIA.

Culture	Treatment per 100 gm. of soil	Ammonia nitrogen per 100 gm. dry soil		
		Average	Increase due to treatment	Nitrogen in casein recovered as ammonia
	Mg.	Mg.	Mg.	%
1	None .....	1.33	.....	.....
2	522 CaCO <sub>3</sub> .....	2.59	1.26	.....
3	30 N from casein .....	14.65	13.32	44.10
4	30 N from casein plus 522 CaCO <sub>3</sub> ...	17.08	14.49	48.03

## GROUP B.—FORMATION OF NITRATES.

Culture	Treatment per 100 gm. of soil	Nitrate nitrogen per 100 gm. dry soil			
		At beg.	At end	Increase	Nitrogen recovered as nitrate
	Mg.	Mg.	Mg.	Mg.	%
1	None .....	.5	.78	.28	...
2	522 CaCO <sub>3</sub> .....	.5	3.12	2.62	....
3	14.2 N from (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	.5	.80	.30	....
4	14.2 N from (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> plus 522 CaCO <sub>3</sub> ...	.5	5.95	5.45	20.0
5	14.2 N from casein .....	.5	3.00	2.50	12.8
6	14.2 N from casein plus 522 CaCO <sub>3</sub> .....	.5	5.60	5.10	17.5

*Miami soil.*—A series of experiments were made to test ammonia and nitrate formation from casein. The amount of nitrogen used in ammonification tests, 30 mg. per 100 gm. of soil, was over twice as great as that used in the nitrification tests.

The results of this study are combined in Table VI, which is subdivided into Group A, ammonia formation, and Group B, nitrate formation. From the evidence presented in the table, two facts stand out very prominently. First, the nitrogen of casein ammonifies and nitrifies al-

most as fast with as without calcium carbonate. It is true that calcium carbonate in this test showed a slight increase in ammonia production, while the results of a second experiment gave no increase for liming. Second, the process of nitrification from ammonium sulphate is favored by calcium carbonate. A repetition of the experiment gave similar results. It seems probable that the benefit derived from the use of calcium carbonate is due to its basic properties. The carbonate reacts to neutralize the acids formed in the oxidation of ammonium sulphate.

*Sparta sand.*—The results of the test with Sparta sand are given in Table VII.

Here again, it is apparent that calcium carbonate at the rate of 0.522 per cent, enough to neutralize the active soil acidity, has very little effect on ammonification of casein. As might be expected, calcium carbonate exerts a very decided influence on the formation of nitrates from inorganic nitrogen. Ammonium sulphate in the absence of calcium carbonate did not nitrify, while casein without calcium carbonate gave a gain in nitrates of almost 13 per cent of the total nitrogen in casein. In the presence of calcium carbonate both substances nitrified, the ammonium sulphate more rapidly than the casein. Why nitrification in acid sand should take place under the conditions of this experiment and not in the previous tests (Table III), cannot be explained unless it is assumed that heat has in some way rendered the soil unfit for these nitrifiers. From the evidence, it seems that nitrification tests as performed in solution and in sterilized soil do not always furnish conclusive proof of the presence or absence of the nitrifying bacteria.

*Colby silt loam.*—The study of nitrification in Colby soil was carried out more in detail than the previous experiments. Since one of the main objects of this work is to note the relation between calcium carbonate and bacterial activity, it was arranged to give special consideration to the quantity necessary for optimum activity of the microorganisms. For this reason, the applications of calcium carbonate were made in three amounts. The first represents half enough to neutralize acidity; the second enough to neutralize acidity completely, and the third double enough to neutralize acidity according to the Truog method. A comparison of the effect of calcium carbonate on ammonification and nitrification is shown in Table VIII. In agreement with earlier tests, it will be seen that calcium carbonate has very little effect on the production of ammonia. The addition of calcium carbonate with ammonium sulphate caused a decided increase in nitrification. It is evident from the data of the table that the maximum nitrification after 3 weeks is reached with the largest amount of calcium carbonate.

In the casein series calcium carbonate produced the opposite effect. The formation of nitrates from casein in this soil seems to bear an in-

verse proportion to the amount of calcium carbonate applied. In the absence of calcium carbonate, casein nitrified almost three times as fast as ammonium sulphate.

TABLE VIII.  
RATE OF NITRIFICATION IN COLBY SILT LOAM.

January 18—January 21.  
GROUP A.—FORMATION OF AMMONIA.

Culture	Treatment per 100 gm. of soil	Ammonia nitrogen per 100 gm. dry soil		
		Average	Increase due to treatment	Nitrogen in casein recovered as ammonia
	Mg.	Mg.	Mg.	%
1	None .....	4.38	.....	.....
2	510 CaCO <sub>3</sub> .....	4.34	.....	.....
3	1021 CaCO <sub>3</sub> .....	3.68	.....	.....
4	2042 CaCO <sub>3</sub> .....	4.10	.....	.....
5	30 N from casein .....	14.45	11.08	33.6
6	30 N from casein plus 510 CaCO <sub>3</sub> ..	16.22	11.88	39.6
7	30 N from casein plus 1021 CaCO <sub>3</sub> ..	15.86	12.18	40.6
8	30 N from casein plus 2042 CaCO <sub>3</sub> ..	16.02	11.92	39.7

November 24—December 16.  
GROUP B.—FORMATION OF NITRATES.

Culture	Treatment per 100 gm. of soil	Nitrate nitrogen per 100 gm. dry soil			
		At beg.	At end	Increase	Nitrogen recovered as nitrate
	Mg.	Mg.	Mg.	Mg.	%
1	None .....	3.57	4.13	.56	.....
2	510 CaCO <sub>3</sub> .....	3.57	5.30	1.73	.....
3	1021 CaCO <sub>3</sub> .....	3.57	6.70	3.13	.....
4	2042 CaCO <sub>3</sub> .....	3.57	7.10	3.53	.....
5	14.2 N from (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	3.57	6.50	2.93	16.6
6	14.2 N from (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> plus 510 CaCO <sub>3</sub> ..	3.57	11.90	8.33	46.0
7	14.2 N from (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> plus 1021 CaCO <sub>3</sub> ..	3.57	13.80	10.23	49.6
8	14.2 N from (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> plus 2042 CaCO <sub>3</sub> ..	3.57	14.80	11.28	54.5
9	14.2 N from casein .....	3.57	10.80	7.23	46.9
10	14.2 N from casein plus 510 CaCO <sub>3</sub> .....	3.57	11.90	8.33	46.1
11	14.2 N from casein plus 1021 CaCO <sub>3</sub> ....	3.57	12.50	8.93	40.6
12	14.2 N from casein plus 2042 CaCO <sub>3</sub> ....	3.57	8.30	5.03	10.5

*Peat.*—The conditions that obtain in peat are so very different from those of the heavier soils that it is difficult to get any comparable data. The calcium carbonate in Group A was added in an amount great enough to neutralize half of the soil acidity. In Group B enough was applied to neutralize all of the acidity.

A glance at Table IX shows that the addition of calcium carbonate greatly benefited ammonia and nitrate formation. Here again, casein nitrified without the addition of any basic substance. In the presence of

calcium carbonate an increase in nitrates from casein is noted, but this is not nearly so great as the gain in nitrates from ammonium sulphate plus calcium carbonate.

TABLE IX.  
RATE OF NITRIFICATION IN PEAT (WYEVILLE).

January 19—January 22.

GROUP A.—FORMATION OF AMMONIA.

Cul- ture	Treatment per 100 gm. of soil	Ammonia nitrogen per 100 gm. dry soil		
		Average	Increase due to treatment	Nitrogen in casein recovered as ammonia
	Mg.	Mg.	Mg.	%
1	None .....	3.03	.....	.....
2	855 CaCO <sub>3</sub> .....	5.69	2.66	.....
3	30 N from casein .....	8.88	5.85	19.5
4	30 N from casein plus 855 CaCO <sub>3</sub> ..	21.65	15.96	53.2

May 8—June 6.

GROUP B.—FORMATION OF NITRATES.

Cul- ture	Treatment per 100 gm. of soil	Nitrate nitrogen per 100 gm. dry soil			
		At beg.	At end	In- crease	Nitrogen recovered as nitrate
	Mg.	Mg.	Mg.	Mg.	%
1	None .....	0.52	2.46	1.94	....
2	21.7 N from (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.52	1.86	1.34	....
3	21.7 N from (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> plus 1710 CaCO <sub>3</sub>	0.52	16.74	16.22	65.7
4	20.3 N from casein .....	0.52	6.79	6.27	21.3
5	20.3 N from casein plus 1710 CaCO <sub>3</sub> .....	0.52	12.52	12.02	49.6

THE EFFECT OF CALCIUM CARBONATE ON AMMONIFICATION AND NITRIFICATION IN COLBY SILT LOAM.

The retarding effect of calcium carbonate on nitrification in certain soils has been reported by Beckwith and his associates. They found the decrease in nitrate nitrogen to be greater with blood-meal than with ammonium sulphate; moreover, that the nitrifying bacteria are influenced by the reaction of the soil; acidity inhibits and likewise "Too much calcium carbonate does not seem best for their growth" (2).

A study of the influence of calcium carbonate on nitrification was undertaken. Two experiments were carried out, using casein and gelatin. The tests were similar in every respect except in the amount of organic nitrogen employed. Periodic determinations were made of the ammonia and nitrates. In one case 15 mg. were used, in the other 30 mg. The procedure was the same as in the foregoing experiments. It seems that the lower nitrate content in the presence of calcium carbonate may be due to one or two factors. First, calcium carbonate may be directly harmful

to the growth of the nitrifying organisms in this soil type; second, calcium carbonate may retard nitrate accumulation by stimulating the growth of those organisms that feed on nitrates.

If calcium carbonate in the amount employed in the preceding experiments is injurious to the nitrifying bacteria, it seems that the decrease in nitrates should be most noticeable soon after it is applied. In order to test this, a series of experiments were planned in which it was arranged to study nitrification at varying intervals.

*Fifteen milligrams of nitrogen.*—In all, five determinations were made. The results of this experiment are presented in Table X.

TABLE X.  
RATE OF NITRIFICATION IN COLBY SILT LOAM.  
June 19—August 13.

Culture	Treatment per 100 gm. of soil	Time after	Nitrate nitrogen per 100 gm. of dry soil				
			Control	Casein		Gelatin	
			Average	Average	Nitrogen recovered as nitrate	Average	Nitrogen recovered as nitrate
	Mg.	Days	Mg.	Mg.	%	Mg.	%
1	None .....	Beg	2.04	2.02	....	1.96	....
2	1021 CaCO <sub>3</sub> ...	Beg.	2.12	2.12	.. .	2.07	....
3	None .....	8	3.79	5.91	14.1	5.98	14.6
4	1021 CaCO <sub>3</sub> ...	8	4.99	9.89	32.6	11.64	44.3
5	None .....	14	4.25	7.49	21.6	7.10	19.0
6	1021 CaCO <sub>3</sub> ...	14	5.32	13.14	52.1	12.97	51.0
7	None .....	28	5.47	12.99	50.1	11.61	40.9
8	1021 CaCO <sub>3</sub> ...	28	14.55	20.36	38.7	21.09	43.6
9	None .....	42	7.01	15.69	58.0	13.87	45.7
10	1021 CaCO <sub>3</sub> ...	42	16.35	24.21	52.4	22.89	43.6
11	None .....	56	7.82	16.61	58.6	16.87	60.3
12	1021 CaCO <sub>3</sub> ...	56	18.18	25.78	50.6	24.54	42.4

No study of ammonia formation was made in the first experiment. From the data of the tests, it will be seen that calcium carbonate greatly stimulated the formation of nitrates from casein and gelatin. This was most marked in analyses after 8 to 14 days. For example, as compared with control, the percentage gain on the eighth day for casein is 231, for gelatin 303. Six days later the relative increase in the calcium carbonate series was not so great. From the fourth week until the end of the experiment, the percentage of nitrogen recovered as nitrate from casein in the calcium carbonate series was below that of the unlimed series.

The beneficial effect of calcium carbonate on the accumulation of nitrates in the soil to which no nitrogen was added was noticeable after 8 days. From then until the end of the experiment in the limed soils, there was a gradual increase in amount of nitrate nitrogen. After 8 weeks the calcium carbonate controls contained more than double as much nitrogen as the blanks.



*Thirty milligrams of nitrogen.*—As a check on the results given in Table X, a similar experiment was planned in which double the amount of organic nitrogen was used. If the data of Table X are correct, calcium carbonate should cause an increase at first in nitrification and later a decrease. In view of the larger amount of organic nitrogen added in this test, it is probable that the period of decrease will not be noted until much later.

TABLE XI.  
RATE OF NITRIFICATION IN COLBY SILT LOAM.

April 1—April 9.

GROUP A.—FORMATION OF AMMONIA.

Cul- ture	Treatment per 100 gm. of soil	Time after	Ammonia nitrogen per 100 gm. dry soil from casein		Ammonia nitrogen per 100 gm. dry soil from gelatin	
			Average	Nitrogen in casein rec'd as ammonia	Average	Nitrogen in gelatin rec'd as ammonia
	Mg.	Days	Mg.	%	Mg.	%
1	None .....	2	11.37	37.9	8.59	28.6
2	1021 CaCO <sub>3</sub> .....	2	14.35	47.8	10.92	36.4
3	None .....	4	16.82	56.0	11.60	38.6
4	1021 CaCO <sub>3</sub> .....	4	21.84	72.8	14.49	48.3
5	None .....	6	19.35	64.5	14.68	48.9
6	1021 CaCO <sub>3</sub> .....	6	24.22	80.7	18.34	61.6
7	None .....	8	20.68	68.9	13.00	43.3
8	1021 CaCO <sub>3</sub> .....	8	24.29	80.9	15.39	51.3

April 1—June 26.

GROUP B.—FORMATION OF NITRATES.

Cul- ture	Treatment per 100 gm. of soil	Time after	Nitrate nitrogen per 100 gm. of dry soil				
			Control	Casein		Gelatin	
			Average	Average	Nitrogen recovered as nitrate	Average	Nitrogen recovered as nitrate
	Mg.	Days	Mg.	Mg.	%	Mg.	%
1	None .....	Beg.	2.10	1.93	.....	1.93	.....
2	1021 CaCO <sub>3</sub> ...	Beg.	2.20	1.92	.....	1.92	.....
3	None .....	8	2.77	4.37	5.33	3.93	3.86
4	1021 CaCO <sub>3</sub> ...	8	3.80	8.75	16.50	8.75	16.50
5	None .....	14	5.96	7.19	4.10	7.95	4.63
6	1021 CaCO <sub>3</sub> ...	14	11.03	21.50	34.90	19.80	29.20
7	None .....	28	7.18	18.01	36.40	17.36	33.60
8	1021 CaCO <sub>3</sub> ...	28	11.34	25.67	47.76	23.33	39.96
9	None .....	42	8.95	22.43	44.90	20.47	38.40
10	1021 CaCO <sub>3</sub> ...	42	12.43	32.59	67.20	27.57	50.40
11	None .....	56	8.00	21.96	46.50	24.18	53.90
12	1021 CaCO <sub>3</sub> ...	56	15.00	31.65	55.50	30.79	52.90

A glance at the data in this table confirms the foregoing statement. The maximum nitrate content is not reached until after the eighth week. Ammonification takes place very rapidly in this soil type, reaching a

maximum at the end of the sixth day. Calcium carbonate increased the rate of ammonia formation. The subsequent decrease in the gelatin series on the eighth day may be due to an oxidation of the ammonia to nitrate.

In order to see if the reduction in nitrates is accompanied by a loss in nitrogen, an attempt was next made to measure the ammonia evolved from the limed and the unlimed soil. The results indicated that only a very slight amount of nitrogen escapes as free ammonia. Apparently calcium carbonate and casein in the amounts employed did not cause an appreciable loss of nitrogen as free ammonia.

Since the results of ammonia determinations failed to explain the loss of nitrate nitrogen, a new series of tests was made. In this case, total nitrogen analyses were made at the beginning and at the end of the experiment. The results of analyses showed that the carbonate of lime produced no loss of nitrogen. In this soil type the injury from calcium carbonate is not a result of any detrimental effect of calcium carbonate on nitrifying bacteria. Calcium carbonate without casein or gelatin always benefited nitrification. Moreover, no injury from calcium carbonate was noted until about 50 per cent of the nitrogen was converted into nitrates. It seems that the decrease in nitrate nitrogen from the use of calcium carbonate and organic nitrogen is due to a conversion of nitrate nitrogen into an organic form and not to any injurious effect on the nitrifying organisms. Since the conditions are favorable for bacterial development, it seems probable that an increase in nitrate-assimilating organisms may cause a decrease in nitrate content of the soil.

According to Miller (7), carbonate of lime causes a slight increase in number of soil bacteria. The gain is not noticeable at first. However, after 42 days he found double as many bacteria in the treated as in the untreated soil. Somewhat similar data were obtained with Colby silt loam soil. In this case, 41 days after treatment the limed series contained almost double as many bacteria. Later counts showed even a greater gain.

In a new test it was arranged to measure nitrate formation and number of bacteria at varying intervals. The experiment was set up September 16 and ended December 22. According to Arnd, carbonate of lime benefits the denitrifying flora (1). The nature of the agent that reduces the nitrate content of soil is indicated from the data in Table XII.

From the data it will be seen that at first calcium carbonate greatly benefits the rate of nitrate formation from gelatin. Later the reverse is true, there is a decrease in nitrate content in the calcium carbonate series. The results of plate counts offer an explanation for this phenomenon. Wherever the soils were treated with calcium carbonate and organic nitrogen, the number of bacteria increased enormously. The gain in total number of bacteria in this case far exceeded the gain with carbonate of

lime alone. Apparently the great number of organisms in these soils results in a reduction of nitrate nitrogen. As regards the effect of different amounts of organic nitrogen, there is apparently no difference between 15 and 30 mg. of gelatin. From the results obtained with calcium nitrate in the presence of calcium carbonate, it appears that in the absence of added organic matter, the reduction of nitrates in Colby silt loam soil does not take place so rapidly.

Plate I shows the relative number of colonies on Heyden agar plates at the time of the third count, 41 days after treatment. The same amount of soil was used in each petri dish. A glance at the colonies indicates very

TABLE XII.  
EFFECT OF VARIOUS SUBSTANCES ON THE RATE OF NITRIFICATION IN COLBY SILT LOAM.

September 16—December 22.

GROUP A.—NITRATE FORMATION.

Treatment per 100 gm. of soil		Time in	Nitrate nitrogen in 100 gm. of soil								
			Control Average	Gelatin 30 mg.		Gelatin 15 mg.		Calcium nitrate 15 mg. and gelatin 15 mg.		Calcium nitrate 30 mg	
				Aver- age	Nitri- fied	Aver- age	Nitri- fied	Aver- age	Nitri- fied	Aver- age	Nitri- fied
Mg	Days	Mg.	Mg.	%	Mg.	%	Mg.	%	Mg.	%	
None	7	6.6	9.1	8.4	8.9	15.2	21.3	—2.0	36.51	99.7	
2040 CaCO <sub>3</sub> ....	7	8.7	13.0	14.3	13.9	34.6	26.3	17.8	37.7	96.8	
None	21	7.9	21.1	43.8	16.5	57.3	28.7	38.2	37.6	99.0	
2040 CaCO <sub>3</sub> ....	21	13.7	30.9	57.4	20.5	45.1	38.2	63.0	42.0	94.3	
None	41	10.0	45.2	84.0	22.7	84.1	39.1	93.7	42.4	107.8	
2040 CaCO <sub>3</sub> ....	41	19.8	43.8	80.0	30.2	69.3	48.5	91.3	51.0	104.0	
None	69	10.4	35.7	84.3	25.3	98.6	37.0	77.3	40.0	98.7	
2040 CaCO <sub>3</sub> ....	69	20.4	43.1	75.6	33.6	87.3	48.1	84.0	48.5	93.7	
None	77	11.4	34.6	77.3	24.2	85.3	37.3	72.6	40.9	98.3	
2040 CaCO <sub>3</sub> ....	77	23.7	45.0	71.0	34.0	68.7	47.6	59.3	50.0	87.7	

GROUP B.—NUMBER OF BACTERIA.

Treatment per 100 gm. of soil	Time in	Bacteria per gram of soil <sup>1</sup>			
		Control	Gelatin 30 mg.	Gelatin 15 mg.	Calcium nitrate 15 mg. and gelatin 15 mg.
Mg.	Days				
None	7		4,187	10,670	6,078
2040 CaCO <sub>3</sub> ....	7		4,862	20,666	14,047
None	21		9,177	25,769	9,297
2040 CaCO <sub>3</sub> ....	21		15,184	32,489	29,484
None	41		7,158	8,104	7,564
2040 CaCO <sub>3</sub> ....	41		13,507	66,587	27,688
None	69		3,512	5,132	4,457
2040 CaCO <sub>3</sub> ....	69		14,182	31,875	33,091
None	77		810	675	945
2040 CaCO <sub>3</sub> ....	77		10,940	24,312	21,070

<sup>1</sup> Thousands omitted.

clearly the effect of gelatin and calcium carbonate on the multiplication of bacteria.

A comparison of the number of bacteria and the amount of nitrate nitrogen in limed soils shows that to a certain degree these two factors are reciprocal. The greater the number of bacteria the lower the nitrates. Since the increase in number of bacteria in certain of these soils results in greater assimilation of nitrates, it seems that this same fact should be noted in nutrient solutions containing nitrate nitrogen. Accordingly 100-c.c. portions of Giltay's solution in 150-c.c. Erlenmeyer flasks were inoculated with equivalent amounts of soil taken from the cultures of the previous experiment. The samples were drawn at the end of the 69-day period. After inoculation, the culture solutions were incubated for 36 hours at 28°C. Nitrate determinations, as well as plate counts, are given in Table XIII.

TABLE XIII.  
EFFECT OF LIME CARBONATE ON NUMBER OF BACTERIA AND  
NITRATE REDUCTION.

No.	Treatment of the inocula	Mg. of nitrate nitrogen per 100 c.c. of solution			Nitrogen rec'd as nitrates	Bacteria added per gm. of soil <sup>1</sup>
		Begin'g	End	Loss		
	Mg.	Mg.	Mg.	Mg.	%	
1	None .....	12.4	10 00	2.40	80.0	3,512
2	2040 CaCO <sub>3</sub> .....	12.4	8.02	4.38	64.0	14,182
3	30 gelatin .....	12.4	10.20	2.20	81.0	5,133
4	15 gelatin .....	12.4	9.70	2 70	77.6	4,457
5	30 gelatin plus 2040 CaCO <sub>3</sub> .....	12.4	3.06	9 34	24.4	31,875
6	15 gelatin plus 2040 CaCO <sub>3</sub> ... ..	12.4	2.20	10.20	17 2	33,091
7	15 Ca(NO <sub>3</sub> ) <sub>2</sub> nitrogen plus 15 gel atin .....	12.4	9.20	2 20	73 6	5,673
8	15 Ca(NO <sub>3</sub> ) <sub>2</sub> nitrogen plus 15 gel tin plus 2040 CaCO <sub>3</sub> .....	12 4	3.20	8 40	25.6	38,494

<sup>1</sup> Thousands omitted.

The results of these experiments are very interesting. The soils with the highest number of bacteria reduced the nitrates of Giltay's solution most rapidly. A glance at the figures of the last two horizontal columns shows that the number of bacteria and the percentage of nitrate recovered, are inversely proportional. The data furnish additional proof that the organisms in the treated soils take up nitrate nitrogen in their bodies. The loss of nitrates in the presence of calcium carbonate and organic nitrogen may be accounted for in this way.

#### ACCUMULATION OF NITRATES IN VARIOUS SOILS.

It has been shown repeatedly that if soil is protected from leaching, a part of the nitrogenous substances will be converted into ammonia and

later into nitrates. Under field conditions ammonia rarely occurs in large quantities. It is nitrified almost immediately: therefore the accumulation of nitrates in uncropped soil will depend to a great degree on the rate of ammonification (8). In this work with the various soil types only the amount of nitrate nitrogen was determined. No attempt was made to measure ammonification. In order to secure maximum accumulation of nitrates, the experiment was allowed to run for a long period of time.

*Accumulation of nitrates.*—Fresh samples of soil were collected, carefully mixed and filled into 2-gallon jars. The jars were kept in the greenhouse at a temperature of about 28°C. and watered at regular intervals with distilled water. All determinations were made from duplicate jars. The average results are presented in Table XIV.

TABLE XIV.  
ACCUMULATION OF NITRATES IN VARIOUS SOILS.  
June 6, 1914—June 6, 1915.

Culture	Soil	Treatment per 100 gm. of soil	Nitrate nitrogen per 100 gm. of dry soil							
			At beg.	4 wks.	8 wks.	12 wks.	16 wks.	24 wks.	32 wks.	52 wks.
		Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.
1	Miami	None .....	1.58	8.33	14.7	13.8	14.0	16.8	21.60	22.80
2	Sand	None .....	0.14	1.66	2.0	1.5	1.6	....	3.40	3.60
3	Sand	177 CaCO <sub>3</sub> .....	0.14	1.50	2.4	1.1	2.0	3.7	14.63	6.19
4	Colby	None .....	0.42	4.35	11.0	...	12.2	12.8	....	25.60
5	Colby	161 CaCO <sub>3</sub> .....	0.42	2.86	11.5	12.5	12.4	14.4	19.84	31.67
6	Peat	None .....	0.52	8.82	9.7	13.6	....	20.8	20.00	25.14
7	Peat	1710 CaCO <sub>3</sub> .....	0.52	9.90	12.7	17.9	17.7	36.2	135.00	73.44

<sup>1</sup> Denotes second application of calcium carbonate.

The results of Table XIV show the very great accumulation of nitrates which is noticeable in the absence of any added base. In the neutral soil there was a gradual rise in nitrate nitrogen during the entire experiment. The total accumulation of nitrate nitrogen in this soil is somewhat greater than that reported in an earlier publication (5, 6). With the exception of Wyeville peat, the accumulation of nitrates was slower in the acid soils than in the neutral Miami soil. As shown by the figures of the calcium carbonate series, the nitrate continued to accumulate up to the thirty-second week. It has been noted repeatedly that ammonia and nitrate accumulation in uncropped soil will proceed up to a certain amount, when it ceases (3, p. 178; 9). Because of the small gains in nitrate nitrogen towards the latter part of the experiment, calcium carbonate was added again to the soils of jars 3, 5 and 7. The amount in this case was the same as that used in the beginning. If the decreased rate of nitrification is a result of too much acid, then a second application should in all probability cause an increase in nitrates. The figures in the last column furnish proof for this statement. In order

to bring out this point more clearly, acidity determinations were made. The accumulation of nitrates should increase soil acidity, that is, provided the supply of basic substances in soil is low. It is of interest to note the general course of nitrification in acid soils.

*Effect on reaction.*—Table XV gives the results of acidity tests. (Veitch method.)

TABLE XV.  
LIME REQUIREMENT OF SOILS USED FOR NITRATE ACCUMULATION.

Soil	Treatment per 100 gm. of soil Mg.	Calcium carbonate required to neutralize 100 gm. of dry soil	
		At beginning Mg.	At end Mg.
Miami Sand	None . . . . .	Alkaline	Alkaline
	1. None . . . . .	177 8	147.2
	2. (a) Initial 177 $\text{CaCO}_3$ (b) After 32 weeks 177 $\text{CaCO}_3$ . . . . .	. . .	31.6
Colby	1. None . . . . .	161 7	264.4
	2. (a) Initial 161 $\text{CaCO}_3$ (b) After 32 weeks 161 $\text{CaCO}_3$ . . . . .	. . . . .	39.2
Peat	1. None . . . . .	1710 0	1172.0
	2. (a) Initial 1710 $\text{CaCO}_3$ . . . . . (b) After 32 weeks 1710 $\text{CaCO}_3$ . . . . .	. . . . .	Alkaline

It is evident that the initial amount of calcium carbonate added to the various acid soils was insufficient to counteract the acidity. The Miami silt loam, which was well supplied with basic elements at the beginning, still showed a neutral reaction, indicating that ample bases were present to care for any accumulation of acidity.

Sand under ordinary conditions is deficient in carbonates. After a period of 52 weeks this soil showed a lower calcium carbonate requirement than at the beginning of the experiment. This may be explained partly by the fact that distilled water was used throughout the entire period.

The Colby silt loam presented a different situation. Here, after a period of 52 weeks, a marked increase in acidity is evident from the data in Table XV. This increased acidity is most probably caused in part by the large accumulation of nitrate nitrogen. The initial calcium carbonate added was not sufficient to keep the soil in good nitrifiable condition. This is shown in the increased production of nitrate nitrogen after the second application, ranging from 19.84 mg. before to 31.67 mg. after treatment.

The peat soil showed a lower calcium carbonate requirement after the period than before. Here, the nitrate nitrogen content was high. No explanation for this result is offered at this time unless it is assumed that the bacteria have destroyed some of the organic acids. After the second application of calcium carbonate, a marked increase in nitrate nitrogen resulted.

In this connection, it is well to know the percentage of the total nitrogen that was converted into nitrates. The Miami silt loam contained 202.8 mg. of total nitrogen and showed an increase of 21.22 mg. of nitrate nitrogen, or 10.4 per cent of the total nitrogen.

The sand untreated showed an increase of only 3.46 mg. of nitrate nitrogen with 106.8 mg. of total nitrogen or 3.2 per cent. The treated sand showed an increase of 6.05 mg. nitrate nitrogen, or 5.6 per cent. Treatment of sand with calcium carbonate caused an increased production of nitrate nitrogen. The increase in the treated soil was 2.4 per cent greater than in the untreated.

Colby untreated showed an increase of 25.18 mg. of nitrate nitrogen with a total of 281.2 mg. or 8.95 per cent of the total nitrogen. The treated soil showed an increase of 31.25 mg. or 11.1 per cent of the total nitrogen. Calcium carbonate resulted in an increased oxidation of 2.15 per cent of organic nitrogen.

Peat untreated showed an increase of 24.62 mg. of nitrate nitrogen with a total of 1229.6 mg., or 2 per cent. The treated soil showed an increase of 72.92 mg., or 5.9 per cent. Treatment of peat with calcium carbonate stimulated nitrate production. The increase amounted to 3.9 per cent.

It is apparent from the foregoing figures that a large amount of the total nitrogen found in acid soils is converted into nitrate nitrogen. This amount is increased materially where acid soils are treated with calcium carbonate.

#### SUMMARY.

The formation of ammonia from casein takes place so rapidly in acid soils that for several weeks after the nitrogenous substance is added, the production of nitrates is not limited by lack of ammonia. The formation of nitrates in acid sand and acid Wyeville peat takes place very slowly. In acid Colby silt loam or the neutral Miami silt loam, nitrification takes place much more rapidly. The feeble nitrifying power of the sand and peat, as shown by inoculating these soils with an active culture of the nitrifying bacteria, is largely due to the condition of the soil. Apparently the nitrifying flora of Colby silt loam when transferred to a neutral soil is as active in the formation of nitrates as the flora from Miami silt loam.

In the case of the acid soils, it seems that the nature of the compound to be nitrified plays an important part. For example, in acid soils organic nitrogen nitrifies much more rapidly than nitrogen from ammonium sulphate. In non-acid soils the reverse is true, ammonium sulphate nitrifies more rapidly. This is true regardless of the source of the nitrifying bacteria. From the data, it seems that acid soils do not possess a strain of nitrifying bacteria especially resistant to soil acidity.

In the presence of organic nitrogenous substances as casein and gelatin, calcium carbonate did not permanently increase the accumulation of nitrates. For a short interval, one or two weeks, calcium carbonate stimulates nitrate formation; later the reverse is true and there is a decided decrease in the treated series. Apparently the reduction of nitrates is largely due to bacteria. It has been found that in the treated soil there is an enormous multiplication of the nitrate assimilating bacteria.

When stored under conditions that prevent leaching, all of the soils showed a gain in nitrate nitrogen. It seems that in Colby silt loam nitrification increases soil acidity and thus it becomes necessary to add a basic substance in order to keep the process going.

Before the results of the laboratory tests can be applied to field practice, it will be necessary to study nitrate formation on field plots.

Considering the data as a whole, it seems that under laboratory conditions, the beneficial effect of calcium carbonate on plant growth must be accounted for by some processes other than the direct effect on nitrification. The beneficial effect of calcium carbonate on nitrification takes place before higher plants begin to draw heavily on the nitrogen of nitrates. Moreover, the period of rapid accumulation from liming may result in a loss of nitrogen from leaching of the nitrates. The results of field tests should give an answer to these questions.

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## PLATE I.

Colonies of bacteria on Heyden agar plates in nitrification studies of Colby Silt Loam.

Fig. 1.—Control.

Fig. 2.—Control plus  $\text{CaCO}_3$

Fig. 3.—Small amount of Gelatin.

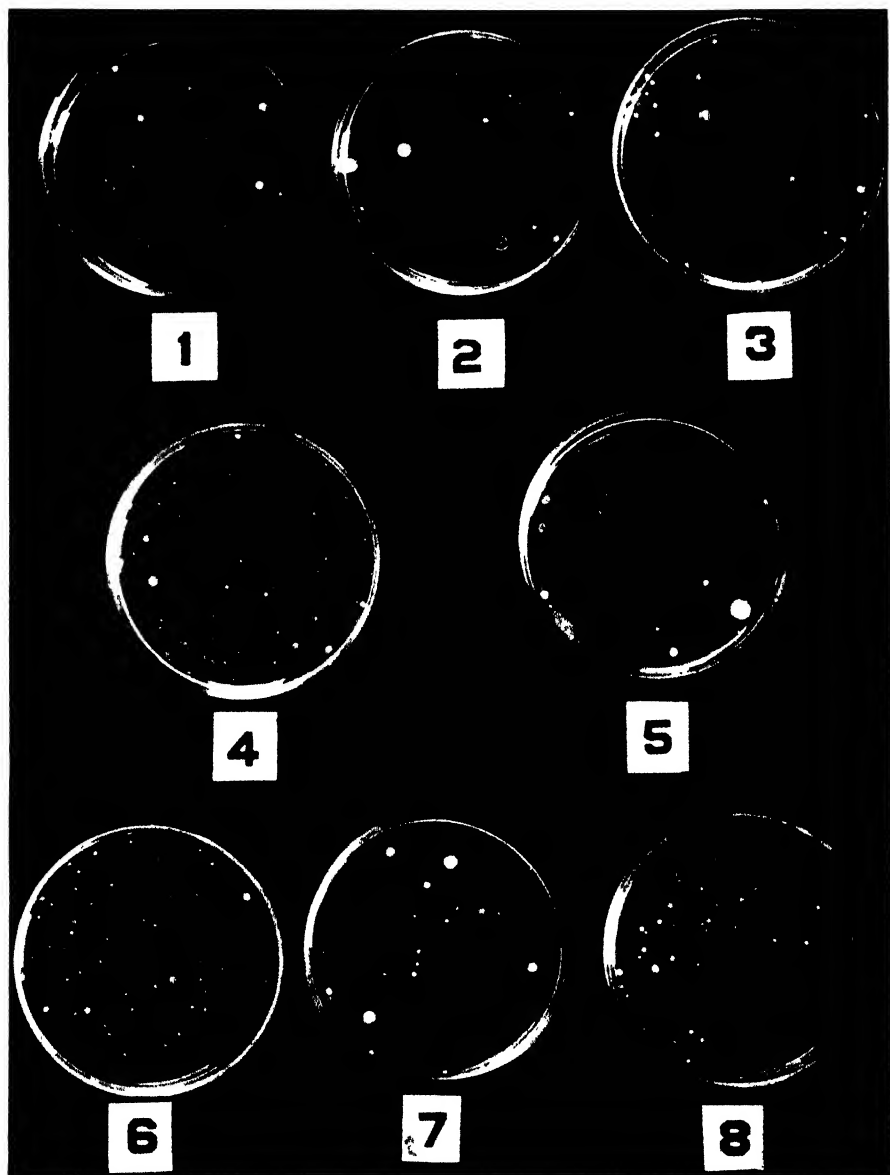
Fig. 4.—Small amount of Gelatin plus  $\text{CaCO}_3$

Fig. 5.—Large amount of Gelatin.

Fig. 6.—Large amount of Gelatin plus  $\text{CaCO}_3$

Fig. 7.—Gelatin plus  $\text{Ca}(\text{NO}_3)_2$

Fig. 8.—Gelatin plus  $\text{Ca}(\text{NO}_3)_2$  plus  $\text{CaCO}_3$





# STUDIES IN SULFOFICATION.<sup>1</sup>

BY

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## I.

Recent experiments (1) have shown that sulfofication or sulfur-oxidation is an important process occurring in field soils. Plants have been found to require considerable amounts of sulfates for their best growth and inasmuch as sulfur is not present in soils in that form but as unassimilable organic and inorganic compounds, it is apparent that the ability of a soil to produce sulfates from these unavailable substances will determine very largely the sulfur-feeding of the crops grown. In other words, the total sulfur content, alone, of a soil will not show the sulfur available for plant growth. The sulfofying or sulfate-producing power of the soil must also be ascertained.

The investigations mentioned besides demonstrating the fact that all soils possess a definite sulfofying power which is determinable in the laboratory, threw considerable light upon the conditions governing the process. Thus it was found that additions of green manure and barnyard manure increased the sulfofying power of the soil, and in general, that the treatment which the soil had undergone influenced considerably its ability to produce sulfates. Furthermore, the optimum moisture content of the soil for the occurrence of the process was found to be 50 per cent of the amount necessary for complete saturation, and the oxidation of sulfur was found to occur to the greatest extent in a mixture of 50 per cent soil and 50 per cent sand, showing the importance of aeration. Again the addition of carbohydrates to the soil was shown to depress sulfofication, the greater the amount added, the greater the depression, and the depression also varied in the inverse ratio to the solubility of the carbohydrates.

A definite laboratory method was devised for determining the sulfofying power of soils and this consisted in the addition of 0.1 gm. of  $\text{Na}_2\text{S}$  or free sulfur, preferably the latter, to 100-gm. quantities of fresh soil, adjustment of the moisture content to the optimum for the soil, and incubation for from 5 to 10 days. The sulfates were then determined by shaking the soil with water for 7 hours in the shaking machine, filtering, precipitating the sulfates with barium chloride, and estimating in the sulfur photometer.

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Studies on the sulfur content of Iowa soils confirm the observations of Hart and Peterson (2) in Wisconsin and Shedd (3) in Kentucky, that on the average much less sulfur than phosphorus was present in the various large soil areas. Some of the sulfur removed from the soil by crops may, of course, be returned by the use of manure, but the amount of manure produced on a livestock farm is quite insufficient, as a general rule, to keep up the sulfur content of the soil, and unless manure is purchased or large amounts of commercial feeds are used, commercial sulfur-containing fertilizers must be applied to maintain the soils permanently fertile.

This does not mean that applications of sulfur fertilizers would prove profitable on Iowa soils at the *present time*, but it does mean that unless different methods of soil treatment are employed than those at present in use, at *some future time*, sulfur will be lacking. In other words for *permanent soil fertility*, the sulfur supply for crops must be considered.

It is known at the present time that the amount of phosphorus present in Iowa soils is low, and in many cases this element may be the limiting factor of growth. Acid phosphate and rock phosphate are the two materials which are available commercially for supplying phosphorus. The former supplies sulfur as well as phosphorus and the question arises whether it may have any superior value because of the sulfur added. The relative merits of the two phosphorus compounds are not yet definitely known, and it is possible that the sulfur content, and also the effect on sulfification, should be considered in selecting the material which should be recommended for remedying deficiencies of phosphorus in soils.

The relative effects of the materials mentioned on sulfification, on ammonification, and on crop yields should also be ascertained. If sulfification and ammonification run parallel, it will be evident that methods of treatment which are of value because of a stimulation of nitrate production will also lead to greater sulfate production. If crop yields and sulfification are similarly affected, it may be that the effects of the materials are largely due to the sulfur factor.

Therefore, the following experiment was planned to throw some light upon the problem of the relative effects of gypsum, acid phosphate, rock phosphate, alone and with gypsum, and mono-calcium phosphate on sulfification, on ammonification and on the yields of oats in pots in the greenhouse.

#### THE PLAN OF THE EXPERIMENT.

The soil used in this work was a Carrington Loam, high in organic matter and having a basic reaction. When analyzed, it was found to contain 911 pounds of phosphorous and 2487 pounds of sulfur per acre of two million pounds of surface soil. This sulfur content was very high, much higher than that of any of the samples of Iowa soils whose analyses were given in the bulletin already referred to.

The results secured were undoubtedly modified considerably because of the presence of so much sulfur in the soil used. The use of gypsum for instance could hardly be expected to show any effect and all of the applications would probably exert much more influence on sulfofication in a soil lower in sulfur.

This soil was evidently somewhat abnormal for Iowa conditions, and hence the results should not be interpreted as of more than technical and special interest.

Twenty-four 4-gallon pots were filled with the soil described which was in an air-dried condition, 27.6 pounds being placed in each pot.

The special treatments were as follows:

<i>Pots.</i>	<i>Treatment.</i>
1- 2.	Check.
3- 4.	24.7 lbs. calcium sulfate per acre.
5- 6.	70.5 lbs. mono-calcium phosphate per acre.
7- 8.	300 lbs. acid phosphate per acre.
9-10.	1000 lbs. rock phosphate per acre.
11-12.	1000 lbs. rock phosphate + 24.7 lbs. $\text{CaSO}_4$ per acre.
13-14.	Check.
15-16.	24.7 lbs. $\text{CaSO}_4$ per acre.
17-18.	70.5 lbs. $\text{CaH}_4 (\text{PO}_4)_2$ per acre.
19-20.	300 lbs. acid phosphate per acre.
21-22.	1000 lbs. rock phosphate per acre.
23-24.	1000 lbs. rock phosphate + 24.7 lbs. $\text{CaSO}_4$ per acre.

Pots 13 to 24 were seeded to oats and the remainder were kept bare for bacteriological tests.

The applications were based on actual field conditions, the 300 pounds of acid phosphate and 1000 pounds of rock phosphate forming the basis of the additions. The acid phosphate was analyzed for phosphorus and sulfur and showed a content of 5.2 per cent of phosphorus and 1.994 per cent of sulfur. The applications of calcium sulfate and mono-calcium phosphate made to the soil were calculated, so that the same amounts of sulfur and phosphorus, respectively, as were applied in the acid phosphate should be added.

The rock phosphate contained 5.85 per cent of phosphorus and hence considerably more phosphorus was applied than in the case of the acid phosphate, but the amounts of both materials used were those commonly employed on the farm, and hence a fair comparison was provided. The variation in available phosphorus, of course, accounts for the difference in the amounts applied.

The optimum moisture content of the soil was determined and after the pots were filled, distilled water was applied to bring all the soils up to



that content. The pots were then weighed and during the continuance of the experiment the moisture content was maintained by additions of distilled water to weight.

The oats were harvested just prior to maturity, the green and dry weights secured and the nitrogen content determined.

Samples were drawn for bacteriological tests every two weeks, the sulfates present were determined, the moisture content ascertained and tests for sulfofying power by the free-sulfur-fresh-soil method, previously described, and for ammonification by the casein-fresh-soil method and the dried-blood-fresh-soil method were carried out.

The usual precautions were observed in sampling to secure uncontaminated samples. The sulfate determinations were made by shaking the soil with water for 7 hours in the shaking machines as usual. The ammonia determinations were made by the magnesium-oxide method.

The experiment was begun on January 11th, and the samples were drawn on January 26th, February 9th, February 23rd, March 9th and March 23rd.

#### THE SULFATES PRESENT AT SAMPLING

The amounts of sulfates present in the soils at the various samplings are given in Table I, and the average contents under the different treatments are calculated.

On examining the table it appears that there was little variation in sulfate content at the different samplings. The amounts added were very small and evidently the method used in the subsequent determinations was not sufficiently accurate to show them.

There are some indications in all the samplings, except the last, of a depression in sulfate content in the treated soils, but the differences were too small to be distinctive. The later sulfofying tests showed increases in sulfofying power, due to treatment, and hence it would hardly be reasonable to assume any depression in sulfate content in this case. The variations in results should, therefore, probably be regarded as due to the method of determination and as indicating the absence of any effect of the materials added, rather than as distinctive differences.

It is apparent, however, that the variations in sulfate content from one sampling to the next were very slight, much smaller than is usually the case with nitrates. There are such variations, however, that the conclusion seems justified that sulfate production and assimilation vary in much the same way that nitrate production and assimilation vary. That is, there may be an accumulation up to a certain point which is followed by increased assimilation and hence a decrease in the amount present. In the field, of course, there are losses in sulfates by leaching and assimilation by plants, just as in the case of nitrates, but in these experiments there was no leaching and no plants grown and hence the differences

were due to variations in production and assimilation by bacteria. There are evidently certain sulfate-assimilating bacteria which may become very active in the presence of abundance of sulfates and whose activity declines as the amounts of sulfates present are used up.

It will be left for future experiments to learn more of these sulfate-assimilating bacteria. Their activities may be a source of removal of sulfates from the use of crops, but it is more probable that they would be regarded as a means of preserving sulfates in the soil and preventing the loss by leaching. Sulfates which are used by the assimilating bacteria would later become available again for plant growth, and hence at times of too large sulfate production for the needs of crops. These bacteria would prove of much value in preventing losses by leaching.

TABLE I.  
SULFUR AS SULFATES PRESENT AT TIME OF SAMPLING.

Pot. No.	Treatment	Lab. No.	1st Samples		2nd Samples		3rd Samples		4th Samples		5th Samples	
			Mg. S. as $\text{SO}_4$	Av. for Treatment	Mg. S. as $\text{SO}_4$	Av. for Treatment	Mg. S. as $\text{SO}_4$	Av. for Treatment	Mg. S. as $\text{SO}_4$	Av. for Treatment	Mg. S. as $\text{SO}_4$	Av. for Treatment
1	Check .....	1	4.98		6.22		5.95		5.20		5.36	
		2	5.07		7.27		5.81		5.71		4.48	
2	Check .....	3	4.88		7.27		6.87		6.53		6.68	
		4	5.17	5.02	7.27	7.00	7.28	6.50	6.29	5.93	6.52	5.75
3	$\text{CaSO}_4$ .....	5	3.91		5.81		6.36		5.88		5.84	
		6	4.75		5.61		6.36		6.12		6.92	
4	$\text{CaSO}_4$ .....	7	3.87		5.40		5.61		5.88		5.36	
		8	5.17	4.43	5.71	5.64	5.71	6.01	5.61	5.87	5.52	5.91
5	$\text{CaH}_4(\text{PO}_4)_2$ .....	9	4.83		6.36		7.21		6.12		6.84	
		10	4.83		7.82		7.14		6.46		6.56	
6	$\text{CaH}_4(\text{PO}_4)_2$ .....	11	4.54		5.61		5.11		4.93		5.68	
		12	5.17	4.84	5.34	6.28	5.71	6.44	4.59	5.52	5.68	6.19
7	Acid Phos. ....	13	4.70		5.27		6.87		5.24		6.28	
		14	5.37		5.44		6.02		5.78		6.60	
8	Acid Phos. ....	15	4.83		4.56		5.34		4.83		6.12	
		16	5.17	5.02	4.83	5.02	5.27	5.89	5.24	5.27	6.33	6.33
9	Rock Phos. ....	17	3.64		4.25		4.56		4.69		5.52	
		18	4.39		4.18		4.49		4.69		5.12	
10	Rock Phos. ....	19	3.51		4.25		4.39		4.96		5.36	
		20	4.70	4.02	4.35	4.26	4.56	4.49	4.76	4.77	5.52	5.38
11	R'k Ph. + $\text{CaSO}_4$	21	4.99		5.27		6.46		7.07		6.84	
		22	5.17		5.44		6.36		6.60		6.84	
12	R'k Ph. + $\text{CaSO}_4$	23	3.79		5.40		6.53		5.20		5.76	
		24	4.88	4.71	5.40	5.37	6.38	6.43	5.44	6.07	6.08	6.38

It is evident also from these results that sulfates do not accumulate in soils any more than nitrates do. They seem to be subject to much the same influences as nitrates, and this fact suggests that sulfate production and also sulfate assimilation are very closely related, respectively, to nitrate production and nitrate assimilation, and that the influence of certain known factors on the nitrogen processes may be the same on the sulfur processes.

## THE SULFOFICATION TESTS.

The samples drawn on the dates given previously, were tested for their sulfofying power according to the method described,—the free-sulfur-fresh-soil method.

The results secured at the various samplings are given in Tables II, III, IV, V and VI and the average results for the different treatments appear in Table VII.

TABLE II.  
PER CENT OF ADDED SULFUR, SULFOFIED, 1st SAMPLES.

Pot No.	Treatment	Lab No.	Mg. S. as $\text{SO}_4$	Av. Mg. S. as $\text{SO}_4$	Mg. S as $\text{SO}_4$ in soil after incubation	Av. Mg. S. as $\text{SO}_4$	Mg. S as $\text{SO}_4$ from S. added	% S. Sulfofied for each treatment
1	Check .....	1	lost		7.75			
		2	lost		7.36	7.55		
2	Check .....	3	38.76		8.46			
		4	36.72	36.74	8.23	8.33	28.40	28.40
3	$\text{CaSO}_4$ .....	5	43.69		7.47			
		6	42.84	43.26	7.47	7.47	35.79	
4	$\text{CaSO}_4$ .....	7	43.69		7.34			
		8	45.90	44.78	7.34	7.34	37.44	36.60
5	$\text{CaH}_4(\text{PO}_4)_2$ .....	9	40.46		7.92			
		10	42.50	41.48	8.09	8.00	33.48	
6	$\text{CaH}_4(\text{PO}_4)_2$ .....	11	48.62		7.27			
		12	41.48	45.05	7.41	7.34	37.71	35.60
7	Acid Phos. ....	13	42.50		7.75			
		14	41.99	42.24	7.75	7.75	34.49	
8	Acid Phos. ....	15	38.76		5.88			
		16	36.38	37.57	5.57	5.72	31.85	33.20
9	Rock Phos. ....	17	43.69		5.71			
		18	41.16	42.41	5.71	5.71	36.70	
10	Rock Phos. ....	19	32.64		5.27			
		20	32.64	32.64	5.44	5.35	29.29	33.00
11	R'k Ph. + $\text{CaSO}_4$	21	27.06		7.27			
		22	36.38	36.72	7.58	7.42	29.30	
12	R'k Ph. + $\text{CaSO}_4$	23	36.04		6.22			
		24	34.68	35.36	6.35	6.28	29.08	29.20

On examining the results given in the complete tables, it is found that the duplicate determinations agreed very closely, indicating that the method employed in the estimation of the sulfates was quite satisfactory.

The results from the duplicate pots were not always in perfect agreement, but that is ever the case in greenhouse experiments. Differences in the location of duplicate pots with reference to the glass, seem to exert an important influence on the bacteriological results as well as on the crop yields secured in the greenhouse. Of course, there is the danger of accidental contamination in soils under such abnormal conditions as pertain in the greenhouse, as indicated by the growth of algae, which is frequently observed, the occurrence of molds and possibly also of proto-

zoans. But, in general, the differences in the heat and light relations may account for many of the variations which are encountered.

The results secured in this work from the duplicate pots were as uniform as is usually the case and whatever the causes of the variations may be, it was impossible to ascertain them, and hence the average results must be considered as fairly accurate.

TABLE III.  
PER CENT OF ADDED SULFUR, SULFOFIED, 2ND SAMPLES.

Pot No.	Treatment	Lab No.	Mg. S. as $\text{SO}_4$	Av. Mg. S. as $\text{SO}_4$	Mg. S. as $\text{SO}_4$ in soil after incubation	Av. Mg. S. as $\text{SO}_4$	Mg. S. as $\text{SO}_4$ from S. added	% S. Sulfofied for each treatment
1	Check . . . . .	1	32.30		7.99			
		2	31.11	31.70	8.19	8.09	23.16	
2	Check . . . . .	3	28.07		7.99			
		4	29.75	28.91	8.74	8.36	20.25	21.72
3	$\text{CaSO}_4$ . . . . .	5	31.11		7.28			
		6	31.11	31.11	7.21	7.24	23.87	
4	$\text{CaSO}_4$ . . . . .	7	35.19		6.97			
		8	36.89	36.04	6.87	6.92	29.12	26.50
5	$\text{CaH}_4(\text{PO}_4)_2$ . . . .	9	35.19		8.02			
		10	35.53	35.36	8.40	8.21	27.15	
6	$\text{CaH}_4(\text{PO}_4)_2$ . . . .	11	35.19		5.75			
		12	36.38	35.78	5.75	5.75	30.03	28.60
7	Acid Phos. . . . .	13	28.07		7.28			
		14	30.60	29.33	7.07	7.17	22.16	
8	Acid Phos. . . . .	15	28.96		6.29			
		16	26.69	27.79	5.88	6.08	21.71	21.90
9	Rock Phos. . . . .	17	30.09		5.24			
		18	31.11	30.60	5.20	5.22	25.38	
10	Rock Phos. . . . .	19	31.79		5.34			
		20	29.24	30.01	5.05	5.19	24.82	25.10
11	R'k Ph. + $\text{CaSO}_4$	21	27.20		6.46			
		22	34.85	31.02	6.53	6.49	24.52	
12	R'k Ph. + $\text{CaSO}_4$	23	27.71		6.73			
		24	31.79	29.75	6.29	6.50	23.25	23.90

Each table gives the amounts of sulfates present in the soils after incubation, and upon examining these results and comparing them with the amounts of sulfates which the soils contained at sampling, given in Table I, it appears that the incubation of the soils for 10 days brought about only very slight changes in the sulfate content of the soils. It is evident, therefore, that the amount of sulfates present in soils does not change to any great extent in short periods of time. In other words in the absence of leaching and of assimilation by crops there seems to be somewhat of an equilibrium established between sulfate-production and sulfate-assimilation. At any rate, the sulfate content of soils under these conditions changes so slowly, that tests made within short intervals of time do not seem to show any large differences.

Under field conditions it is quite probable that the differences would be much greater and would appear in a much shorter space of time. In short, it seems extremely doubtful if an equilibrium such as was found here would be established under field conditions in the presence of the disturbing factors of leaching and assimilation by crops. Unless special treatments were followed it would be reasonable to expect that the sulfate content of soils would gradually decline, and such is actually the case in the field. As the total sulfur content becomes less the production of sulfates becomes slower, as has been shown in the sulfonation studies already referred to. Hence under field conditions instead of an equilibrium in sulfates, a gradual decline is found unless special treatments are followed.

TABLE IV.  
PER CENT OF ADDED SULFUR, SULFOFIED, 3RD SAMPLES.

Pot No.	Treatment	Lab No.	Mg. S. as $\text{SO}_4$	Av. Mg. S. as $\text{SO}_4$	Mg. S. as $\text{SO}_4$ in soil after incubation	Av. Mg. S. as $\text{SO}_4$	Mg. S. as $\text{SO}_4$ from S. added	% S. Sulfofied treatment for each
1	Check .....	1	33.66		6.02			
		2	33.32	33.49	5.88	5.95	27.54	
2	Check .....	3	34.85		6.60			
		4	34.85	34.85	6.60	6.60	28.25	27.90
3	$\text{CaSO}_4$ .....	5	39.10		7.28			
		6	39.61	39.35	6.97	7.12	32.23	
4	$\text{CaSO}_4$ .....	7	43.69		5.71			
		8	35.70	39.65	5.88	5.79	33.86	33.00
5	$\text{CaH}_2(\text{PO}_4)_2$ .....	9	34.34		7.44			
		10	35.36	34.85	7.28	7.36	27.49	
6	$\text{CaH}_2(\text{PO}_4)_2$ .....	11	31.79		5.17			
		12	33.66	32.72	5.34	5.25	27.47	27.50
7	Acid Phos. ....	13	32.30		5.20			
		14	31.96	32.13	4.42	4.81	27.34	
8	Acid Phos. ....	15	34.34		4.69			
		16	33.66	34.00	5.13	4.91	29.09	28.20
9	Rock Phos. ....	17	37.23		4.79			
		18	35.36	36.29	4.69	4.74	31.53	
10	Rock Phos. ....	19	41.99		5.00			
		20	38.76	40.37	4.83	4.91	35.46	33.50
11	R'k Ph. + $\text{CaSO}_4$	21	39.95		5.85			
		22	41.99	40.92	6.19	6.02	34.96	
12	R'k Ph. + $\text{CaSO}_4$	23	32.30		5.95			
		24	34.00	30.15	5.95	5.95	27.20	31.00

Upon subtracting the sulfate content of the soils after incubation from the total amount of sulfates found in the tests, the remainder is calculated as per cent of sulfur sulfofied, and these are the figures which show the sulfofying power of the soils.

Turning to Table VII which gives the average percentages of sulfur sulfofied, some interesting facts become evident.

In the first place it is found that the calcium sulfate, even in the small applications made, increased to a marked degree the sulfofying power of the soil. This marked increase occurred at every date of sampling, and bears out the results secured in the earlier experiments already referred to, according to which calcium sulfate in various amounts increased the sulfofying power of the soil used to a large extent, the influence being in direct proportion to the size of the application. Of course, if the amount of sulfate applied were increased beyond a certain point it is probable that no further increase in sulfofication would occur and an actual depression might take place. The interesting feature of the present results is that very small amounts of gypsum, such as may be added to soils in another fertilizing material (acid phosphate), may exert a pronounced influence upon the ability of the soil to produce sulfates.

TABLE V.  
PER CENT OF ADDED SULFUR, SULFOFIED, 4TH SAMPLES.

Pot No.	Treatment	Lab No.	Mg. S. as $\text{SO}_4$	Av. Mg. S. as $\text{SO}_4$	Mg. S. as $\text{SO}_4$ in soil after incubation	Av. Mg. S. as $\text{SO}_4$	Mg. S. as $\text{SO}_4$ from S. added	% S. Sulfofied for each treatment
1	Check .....	1	34.6		6.92			
		2	34.2	34.4	6.92	6.92	27.5	
2	Check .....	3	33.6		5.48			
		4	34.4	34.0	5.76	5.62	28.4	27.9
3	$\text{CaSO}_4$ .....	5	31.6		6.20			
		6	32.0	31.8	6.40	6.30	25.5	
4	$\text{CaSO}_4$ .....	7	41.6		6.32			
		8	45.6	43.5	6.04	6.18	37.4	31.4
5	$\text{CaH}_4(\text{PO}_4)_2$ .....	9	52.2		5.12			
		10	50.0	51.1	5.76	5.44	45.6	
6	$\text{CaH}_4(\text{PO}_4)_2$ .....	11	43.2		5.08			
		12	44.2	43.7	4.96	5.02	38.7	42.1
7	Acid Phos. ....	13	48.8		6.88			
		14	46.6	47.7	6.84	6.86	40.8	
8	Acid Phos. ....	15	47.0		6.72			
		16	46.0	46.5	6.32	6.52	40.0	40.4
9	Rock Phos. ....	17	50.0		5.36			
		18	51.4	50.7	5.16	5.26	45.5	
10	Rock Phos. ....	19	47.0		5.40			
		20	43.8	45.4	5.40	5.40	40.0	42.7
11	R'k Ph. + $\text{CaSO}_4$	21	35.0		7.32			
		22	42.0	39.5	6.92	7.12	32.4	
12	R'k Ph. + $\text{CaSO}_4$	23	40.4		6.40			
		24	39.2	39.8	5.40	5.90	33.9	33.1

In other words, the effects of gypsum may be partly due to a stimulative action as has been supposed as well as to the addition of a plant food constituent. The stimulative action may be of considerable importance on soils which contain sufficient amounts of total sulfur but do not have a rapid enough sulfofying action. In other words if soils are found which

contain fairly large amounts of sulfur but on which crops are not supplied with sufficient sulfates for their best growth, applications of small amounts of gypsum might be sufficient to stimulate sulfofication to such an extent that the sulfur already present in the soil would be sulfified rapidly enough to keep plants supplied with that element.

The mono-calcium phosphate gave considerable increases in sulfofication, and these were especially pronounced at the last two samplings. The increases were very similar to that brought about by the gypsum, varying somewhat from those results, as might be expected. It is apparent that this material exerted some stimulative action on sulfofication, and if it has any effect on crop growth, that effect might be considered to be due

TABLE VI.  
PER CENT OF ADDED SULFUR, SULFIFIED, 5TH SAMPLES.

Pot No.	Treatment	Lab No.	Mg. S. as $\text{SO}_4$	Av. Mg. S. as $\text{SO}_4$	Mg. S. as $\text{SO}_4$ in soil after incubation	Av. Mg. S. as $\text{SO}_4$	Mg. S. as $\text{SO}_4$ from S. added	% S. Sulfified for each treatment
1	Check .....	1	35.4		5.12			
		2	36.6	35.9	5.08	5.10	30.8	
2	Check .....	3	45.6		5.72			
		4	44.6	45.1	5.72	5.72	29.4	30.1
3	$\text{CaSO}_4$ .....	5	36.6		5.88			
		6	47.6	48.1	6.16	6.02	41.1	
4	$\text{CaSO}_4$ .....	7	45.6		5.40			
		8	44.6	45.1	5.40	5.40	39.7	40.4
5	$\text{CaH}_4(\text{PO}_4)_2$ .....	9	45.6		5.88			
		10	43.2	44.4	5.88	5.88	38.6	
6	$\text{CaH}_4(\text{PO}_4)_2$ .....	11	50.0		5.00			
		12	47.6	48.8	4.96	4.98	43.8	41.1
7	Acid Phos. ....	13	40.4		6.24			
		14	40.4	40.4	6.04	6.14	34.3	
8	Acid Phos. ....	15	48.8		5.68			
		16	45.6	47.2	5.56	5.62	41.6	37.9
9	Rock Phos. ....	17	47.0		5.52			
		18	46.6	46.8	5.16	5.34	41.5	
10	Rock Phos. ....	19	45.6		5.16			
		20	43.8	44.7	5.04	5.10	39.6	40.5
11	R'k Ph. + $\text{CaSO}_4$	21	45.6		7.68			
		22	47.6	46.6	6.44	7.06	39.5	
12	R'k Ph. + $\text{CaSO}_4$	23	46.0		6.04			
		24	49.4	47.7	6.04	6.04	41.7	40.6

in part to an increased production of sulfates and not entirely to the phosphorus supplied. The action of this material may be somewhat indicative of the effect of acid phosphate, assuming that the phosphorus in this latter material is in the mono-calcic form which it is in part at least.

The applications of acid phosphate increased the sulfofying power of the soil, but to a smaller extent in practically all cases than either the gypsum or the mono-calcium phosphate alone. It appears, therefore, that

on this soil the combination of the two substances was not as beneficial for sulfofication as either of them alone. Just why this should be the case is difficult to determine. It is probably, however, the result of more complicated bacterial changes brought about by the combined substances, although the other calcium phosphates present in the acid phosphate, such as the dicalcic and tricalcic phosphates may explain the different effects.

An interesting practical point is brought out here, however. The acid phosphate when applied to this soil had a stimulative action on sulfofication, and hence its influence on crop yields, if it exerts any effect whatever, may not be due entirely to the phosphorus which it supplies to the crops in available form or to the sulfate which is supplied, but in part to the increase in sulfate production from the soil. Previous suggestions regarding the value of acid phosphate because of effects on the sulfur feeding of plants are thus confirmed, and it seems reasonable to conclude that on soils deficient in both phosphorus and sulfur, acid phosphate would be a good material to use to supply both deficiencies, increasing the sulfates available for plants both by actual additions and by increased production in the soil.

TABLE VII.  
PER CENT OF ADDED SULFUR, SULFOIFIED FOR EACH TREATMENT  
AT EACH SAMPLING.

Treatment	Samples				
	1st	2nd	3rd	4th	5th
1. Checks . . . . .	28.4	21.7	27.9	27.9	30.1
2. 24.7 lbs. $\text{CaSO}_4$ . . . .	36.6	26.5	33.0	31.4	40.4
3. 70.5 lbs. $\text{CaH}_4(\text{PO}_4)_2$ . .	35.6	8.6	27.5	42.1	41.1
4. 300 lbs. Acid Phosphate . .	33.2	21.9	28.2	40.4	37.9
5. 1000 lbs. Raw Rock Phosphate	33.0	25.1	33.5	42.7	40.5
6. 1000 lbs. Raw Rock Phosphate plus 24.7 lbs. $\text{CaSO}_4$ . .	29.2	23.9	31.0	33.1	40.6

Raw rock phosphate applied at the rate of 1000 pounds to the acre, a normal farm application, increased the sulfofying power of the soil to a greater extent than did the acid phosphate also applied in the customary field amount. The increase was about the same as that exerted by the mono-calcium phosphate. Only one reason suggests itself in explanation of the greater influence of the rock phosphate over the acid phosphate—that it is due to the greater amount of phosphate used. Perhaps the sulfofying bacteria use phosphorus in their growth and the stimulative effect of phosphorus fertilizers on sulfofication is really due to a feeding of the sulfifiers. In such a case, which seems quite probable, the question arises as to in what form the phosphorus is required by the bacteria. Probably it must be in a soluble form when it would be expected that the acid phosphate would give greater increases than the rock phosphate.



It is interesting to consider this effect of rock phosphate on sulfofication from the practical standpoint. If raw rock phosphate will stimulate sulfate production to as large an extent as these results indicate, it may be that the material would be quite as valuable as a phosphorus *and sulfur* fertilizer such as acid phosphate, at least on soils not extremely low in sulfur. In other words, if rock phosphate will stimulate sulfate production from soils sufficiently to supply the needs of crops, it may be unnecessary to use a special sulfur fertilizer except in extreme cases, and the phosphorus fertilizer may be depended on for a dual purpose. Of course, this is assuming that the rock phosphate gives as good effects from the phosphorus standpoint as the acid phosphate, a point which, as has been mentioned, is far from being settled at the present time.

When gypsum was applied with the rock phosphate, increases in sulfofication were noted, but these gains were smaller than those secured with the rock phosphate alone and smaller also than those given by the gypsum alone. The increases were about the same as those given by the acid phosphate. It is apparent again, therefore, that the single constituents gave more effect than the two together. In this case, also, just as with the acid phosphate, the cause for this smaller increase with the combined materials is not apparent and may be due to complicated bacterial changes where the two substances were combined. It is evident that on soils not very deficient in total sulfur, rock phosphate alone may prove just as beneficial as when applied with gypsum because of a greater production of available sulfates.

It must be emphasized again that these results apply to this particular soil only and not to soils in general. The soil used in this work was unusually high in sulfur, as has been pointed out, and hence the effects of sulfur fertilizers would be less pronounced than on soils poorer in sulfur. If there are such pronounced effects on the sulfofying power of this particular soil by small applications of the various fertilizing materials, a much greater effect might be expected from the same substances on a soil poorer in sulfur, or a more normal soil.

Therefore, the following conclusions from this work seem entirely justified, and while they apply specifically to this particular soil, they may be found to be of much more general application:

Applications of acid phosphate, of rock phosphate, of gypsum, of rock phosphate and gypsum, and of mono-calcium phosphate increased the sulfofying power of the soil to a considerable extent.

The rock phosphate, mono-calcium phosphate and gypsum gave the largest increases, larger than those given by the mixtures or by the acid phosphate.

Any of these materials, therefore, when applied to the soil in normal field amounts may be expected to increase sulfate production. Their ef-

fects on crops grown, if any, may be due partly at least to this influence on sulfur transformation. It is particularly interesting to note the greater effect of the rock phosphate than of the acid phosphate on sulfofication. On soils not strongly depleted in sulfur, therefore, but deficient in sulfofication, and also in need of phosphorous, it seems possible that the rock phosphate would prove as satisfactory as the more soluble acid phosphate. Crop yields must, of course, prove this point before it can be accepted definitely.

No reason can be assigned for the greater effects on sulfofication of the single constituents over the combinations. They were probably due to complicated bacterial processes which the latter engendered, and about which nothing is known as yet.

#### THE AMMONIFICATION EXPERIMENTS.

The samples drawn on the dates already mentioned were tested for their ammonifying power by the casein-fresh-soil method and the dried-blood-fresh-soil method. The former method was employed at the first

TABLE VIII.  
AMMONIFICATION TESTS.

Pot No.	Treatment	Lab No	Mg. N. as NH <sub>4</sub>	Av. for Treatment	Mg. N. as NH <sub>3</sub>	Av. for Treatment	Mg. N. as NH <sub>4</sub>	Av. for Treatment	Mg. N. as NH <sub>3</sub>	Av. for Treatment	Mg. N. as NH <sub>4</sub>	Av. for Treatment
1	Check . . . . .	1	86.08		82.49		247.2		268.2		212.4	
		2			83.45		235.4		247.5		229.9	
2	Check . . . . .	3	86.46		81.53		277.2		288.7		212.1	
		4		86.27	82.49	82.49	282.6	261.1	280.7	271.3	210.9	216.3
3	CaSO <sub>4</sub> . . . . .	5	84.53		80.57		292.9		309.7		246.5	
		6			82.49		283.2		291.9		236.6	
4	CaSO <sub>4</sub> . . . . .	7	83.76		83.45		292.3		309.7		211.2	
		8		84.14	83.93	82.64	281.4	287.5	294.6	301.5	238.3	233.1
5	CaH <sub>4</sub> (PO <sub>4</sub> ) <sub>2</sub> . . . . .	9	88.01		83.05		292.3		300.1		213.0	
		10			83.45		284.1		295.9		237.9	
6	CaH <sub>4</sub> (PO <sub>4</sub> ) <sub>2</sub> . . . . .	11	89.17		83.93		278.9		276.9		234.6	
		12		88.59	83.45	83.47	271.4	281.7	280.2	288.3	251.5	234.2
7	Acid Phos. . . . .	13	91.87		85.84		306.5		301.2		249.3	
		14			83.45		284.4		289.6		210.3	
8	Acid Phos. . . . .	15	93.03		80.09		282.9		279.7		222.9	
		16		92.45	80.09	82.36	277.2	287.8	282.8	288.3	234.3	228.9
9	Rock Phos. . . . .	17	87.62		83.05		285.1		291.9		212.4	
		18			85.84		305.6		275.6		203.7	
10	Rock Phos. . . . .	19	86.85		82.01		269.0		280.7		211.4	
		20		87.38		83.22	283.4	285.8	276.9	281.3	214.3	210.4
11	R'k Ph. + CaSO <sub>4</sub> . . . . .	21	87.23		83.93		274.5		300.2		226.6	
		22			85.84		272.6		283.5		220.5	
12	R'k Ph. + CaSO <sub>4</sub> . . . . .	23	87.23		85.36		235.9		293.8		217.9	
		24		87.23	87.28	85.60	230.6	257.9	253.4	282.7	210.9	218.9

and second samplings, the incubation period being 3 and 5 days respectively, but the results were not satisfactory, and the remaining tests were made by the dried-blood method. All of the results secured are given in Table VIII.

The duplicates were much more satisfactory where the casein was used, but the effects of the treatments were not clearly pronounced; the difference in ammonifying power of the soil were too small in many cases to be conclusive. The dried-blood results were more definite, but the same difficulty which is usually met with was encountered with them, that is, the impossibility of securing entirely satisfactory duplicate determinations. However, the results given in the table show certain tendencies among the treatments and it will be worth while to call attention briefly to some points which appear more or less definitely.

The calcium sulfate had the greatest effect of any of the materials on ammonification, the mono-calcium phosphate and acid phosphate were about equal in their effect, but lower than that of the calcium sulfate or the raw rock phosphate, and rock phosphate with calcium sulfate had little influence. Practically all the differences between these averages are as great as between duplicates.

The stimulative action of all these materials on ammonification is indicated by the results secured, and there are some relations evident between the ammonification results and the sulfocation tests. Thus in both cases the calcium sulfate exerted the greatest stimulative action of any of the materials used. In the sulfocation results, however, the rock phosphate alone gave practically as large an effect as the gypsum, while in ammonification it had less influence.

Again, in the sulfocation tests the acid phosphate had less effect than either the calcium sulfate or mono-calcium phosphate, while in ammonification it showed less influence than the calcium sulfate, but practically the same as the mono-calcium phosphate. In both cases the mixture of rock phosphate and calcium sulfate gave small influence.

It is impossible to explain these divergences in results, some of which owing to the difficulties encountered in the methods are not as pronounced as they should be, and indeed it is doubtful if the present results should be regarded as conclusively showing any definite differences in effect on ammonification among the various substances used. Not a large enough number of determinations were made and the duplicate results were not in sufficiently satisfactory agreement.

The stimulative action of all the substances on ammonification was, however, indicated, just as was the case with sulfocation, and hence there must be some relationship between the two processes. Of course, the same groups of organisms are not involved in the different processes, but they may belong in the same class because of their requirements for growth, especially their food materials and the most favorable mechanical soil conditions.

The differences noted in the effect of phosphorus fertilizers may have been due to different effects of phosphorus as a food material on the two

groups of bacteria, but as has been pointed out these variations were not distinctive and it is probable that the food material requirements of the different groups are not very dissimilar.

#### THE CROP YIELDS.

The oats were harvested just prior to maturity, and the green and dry weights secured. The crop was analyzed for nitrogen and the removal of nitrogen from the soil in the crop was calculated. All these results are given in Table IX.

TABLE IX.  
THE CROP YIELDS

Pot No.	Treatment	Green Weights Gm	Average	Dry Weight Gm	Average	% N. in Crop	Gm N. in Crop	Average
1	Check	266 00		52 90		2 572	1.3605	
2	Check	262 40	26 2	49 70	51 30	2 423	1 1942	1.2773
3	CaSO <sub>4</sub>	263 55		50 45		2 310	1.1654	
4	CaSO <sub>4</sub>	243 90	253 7	49 00	49 72	2 346	1.1495	1.1574
5	CaH <sub>2</sub> (PO <sub>4</sub> ) <sub>2</sub>	239 60		48 00		2.677	1 2849	
6	CaH <sub>2</sub> (PO <sub>4</sub> ) <sub>2</sub>	271 35	255 4	50 45	49 22	2 201	1 1104	1 1976
7	Acid Phosphate	327 80		62 70		2 699	1.6923	
8	Acid Phosphate	319 90	323 9	63 00	62 85	2 561	1 6134	1.6528
9	Raw Rock Phosphate	300 00		55.00		2 751	1 5121	
10	Raw Rock Phosphate	323 70	311 9	56 50	55 75	2 652	1.4984	1 5052
11	Raw Rock Phos. plus CaSO <sub>4</sub>	305 30		54 90		2.959	1.6244	
12	Raw Rock Phos. plus CaSO <sub>4</sub>	277 40	291 3	51 70	53.30	2 553	1.3200	1 4722

On examining the table it is found that all the applications of phosphorous except the mono-calcium phosphate increased the crop yield. The acid phosphate gave the largest increase, much larger than that given by the raw rock. When the gypsum was applied with the rock phosphate, slightly lower yields were secured than when the rock phosphate alone was used. The difference, however, was slight and should not be considered as indicating any depression from the use of the gypsum.

The gypsum alone and the mono-calcium phosphate gave no effects. The actual average yields were slightly less than that of the check soils, but the differences in the duplicate pots were as great as those between the checks and the treated soils, and hence the results should merely indicate an absence of effect for the treatments.

It will be recalled that the soil used in this work was very low in phosphorus and hence a beneficial effect of the phosphorus fertilizers on crop yields might have been expected. It is evident from these results that when a soil is as low in phosphorus as this one was, applications of phosphorus fertilizers would prove of value. These results also indicate a superior value for the acid phosphate over the rock phosphate. No conclusions applicable to field conditions should be drawn from this single experiment, especially as it was conducted under greenhouse conditions.

The results may merely serve to indicate what *may* occur under field conditions *on this particular soil type*. No attempt has been made, therefore, to calculate the relative cost of applications and the value of the increases, which would be necessary in field tests, in order to arrive at some conclusions regarding the relative values of the applications.

Why the mono-calcium phosphate should not have brought about any increase in yield is not apparent from the results. A slight depression in the crop yield was actually observed, but it was not large enough to be distinctive, as the differences in the duplicate pots were wider than the differences between the check and treated pots, as has been noted. It appears merely, therefore, that the plants were unable to utilize the phosphorus from this compound as well as from acid phosphate. The sulfate present in the acid phosphate could not account for the greater effect of the latter material as the sulfate alone produced no effect on the crop. Possibly the acidity of the mono-calcium phosphate may explain the results, especially as this would have more effect in the absence of the calcium sulfate than where the two occur together in the acid phosphate.

The use of calcium sulfate on this soil was definitely shown to be of no value. This is as might be expected from the fact that the soil was so abnormally high in sulfur. There was evidently an abundance of sulfur present and in the presence of sufficient organic matter and lime, the process of sulfofication proceeded rapidly enough to keep the oats supplied with sulfates. Even in the presence of phosphorus, where a larger growth was secured, the sulfate had no additional effect, showing the absence of any need for sulfates on this soil.

On comparing the results of the sulfofication tests and ammonification tests with the crop yields, it is found that there were some agreements and some discrepancies in the effects of the various treatments. The gypsum exerted the greatest effect on sulfofication and likewise on ammonification, but had no influence on the crop grown. Mono-calcium phosphate likewise gave a considerable increase in sulfofying power and in ammonifying power, but had no effect on the yield of oats. Acid phosphate however increased sulfofication, ammonification and crop yield, the latter to the greatest extent of any material used, and the two former processes to as great an extent as the other substances applied. Rock phosphate increased the crop yield and the sulfofying power of the soil, but had no pronounced effect on ammonification. It is apparent, therefore, that the sulfofying power of a soil may be increased without a corresponding increase in crop yield occurring. As has been mentioned, conclusions should hardly be drawn from the ammonification results, but it seems that other factors might be of greater importance from the crop standpoint than from the standpoint of the transformation of soil nitrogen at least, in greenhouse soils.

In general, these crop yield results show that on this soil, rich in sulfur but poor in phosphorus, phosphate fertilizers produced a pronounced effect, while sulfates had no influence. The supply of sulfur and of nitrogen available for plant growth was evidently sufficient and phosphorus was the limiting factor of growth. Hence the influence of applications of materials merely increasing the supply of nitrates and sulfates was not apparent above the effect of the use of phosphorus.

#### CONCLUSIONS.

This experiment leads, therefore, to the following conclusions:

1. The sulfate content of the soil varied only slightly from one sampling to the next. There were no sudden or striking changes in the amount of sulfates present in the soil, kept fallow in the greenhouse.
2. The sulfate content of soils in the field is subject to the same influences as the nitrate content, but the effects are probably much less pronounced.
3. Calcium sulfate, mono-calcium phosphate, acid phosphate, rock phosphate and rock phosphate plus gypsum increased the sulfofying power of the soil. The sulfate alone and phosphates alone had greater effects than combinations of the two materials as in acid phosphate.
4. All the materials used increased the ammonifying power of the soil, but the differences between the effects of the various substances were not pronounced. The rock phosphate had less effect, however, than the other materials.
5. The sulfofication tests and ammonification tests did not always run parallel, although very similar effects of the materials used, on the two processes were noted.
6. The phosphorus fertilizers, except mono-calcium phosphate, increased the yield of oats, the acid phosphate to a greater extent than the rock phosphate. The sulfate had no effect on the crop yield. Such results were expected on this soil rich in sulfur but deficient in phosphorus. The lack of effect from the mono-calcium phosphate was probably due to the acidity, which was of more effect in the absence of the sulfate than when the two were together as in the acid phosphate.
7. The crop yields, sulfofication and ammonification results were not always parallel. In general it appeared that on this soil increases in sulfofication were not necessarily parallel with increases in yields. The ammonification results were not conclusive but indicate that materials supplying plant food constituents which are lacking in the soil may be of double value because of increases in the production of other plant food constituents in an available form.

## II.

## SERIES I.

## THE PROPER INCUBATION PERIOD FOR TESTS OF SULFOFICATION.

In previous tests of soils for their sulfofying power by the use of free sulfur, which was found to be the best material to use, the incubation period was 10 days. It seemed desirable to ascertain whether this period of incubation allowed the greatest differentiation between soils from different sources and under varying treatments. Shorter periods of incubation were eliminated, as less satisfactory in earlier experiments and hence the tests here were carried out at 7, 10, 12 and 14 day periods.

Five soils, very different as to texture and composition, and thus presumably varying widely in sulfofying power, were selected. Fresh soil was used, being weighed out in 100-gm. quantities in tumblers, 100 mg. of sulfur added to each, and the moisture content of each of the soils adjusted to the optimum for that particular soil. The sulfates produced at the end of the various incubation periods were determined in the usual way.

TABLE X.  
SULFATES PRESENT AFTER DIFFERENT PERIODS OF INCUBATION

No.	Soils	S as SO <sub>4</sub> after 7 da.	Average	S as SO <sub>4</sub> after 10 da.	Average	S as SO <sub>4</sub> after 12 da.	Average	S as SO <sub>4</sub> after 14 da.	Average
1	Heavy black .....	5.72		11.76		7.92		21.47	
	Woodland soil .....	5.12	5.42	6.72	9.24	11.00	9.46	20.54	21.00
2	Typical sand .....	8.20		9.00		9.12		14.00	
	River-bank in sod ....	6.68	7.44	8.56	8.78	9.52	9.32	14.40	14.20
3	Humus Plot 107 .....	5.84		5.00		4.88		6.10	
	Check .....	8.40	7.12	4.00	4.50	5.16	5.02	6.23	6.16
4	Humus Plot 101 .....	9.88		10.80		18.33		30.80	
	Continuous timothy...	10.28	10.08	11.44	11.12	18.67	18.50	30.60	30.70
5	Corn-field soil .....	9.40		6.04		7.20		10.00	
	River terrace .....	8.08	8.74	6.55	6.30	8.56	7.88	10.20	10.10

On examining the results given in Table X, it is apparent that considerably larger amounts of sulfates were produced from the sulfur added, with the longer incubation periods. None of the soils showed more than a trace of sulfates at the beginning of the experiment, so the entire amount found at the end of the incubation period may be considered as produced from the sulfur added.

At the end of the 7-day period, the differences between the various soils were much too small in several cases to be conclusive. After 10 days' incubation, the amounts of sulfates produced were somewhat larger and the ranking of the soils in sulfofying power had changed materially. The duplicate determinations also agreed much better. In 12 days, the differences in sulfofying power had become still more pronounced, but the ranking of the soils was the same.

Again after 14 days' incubation, the variations among the soils were larger, but the ranking of the soils was the same as after the 10 and 12-day periods.

These results indicate, therefore, that when soils are tested for their sulfofying power by the free-sulfur-fresh-soil method, the tests should be incubated for at least 10 days to secure the proper ranking of the soils, and much better results may be secured by incubating the samples for 12 or even 14 days.

The greatest differences between various soils may be obtained by incubating for the longer period, that is, for 14 days.

## SERIES II.

### THE EFFECT OF GYPSUM ON SULFOFICATION.

In the earlier experiments already referred to, gypsum was found to exert a stimulative effect on sulfofication, but the amounts used were rather small and further tests seemed desirable to ascertain whether large applications would show a greater effect or whether they would depress the activities of the sulfofying bacteria. This series was planned to test this point. The soil used was a Marshall silt loam from Lee County, Iowa. It was air-dried, sieved through a twenty-mesh sieve and weighed out as usual. Sulfur in the usual amount and the special quantities of calcium sulfate were then added and thoroughly stirred in. Ten cubic centimeters of a soil infusion, made by shaking 100 gm. of fresh soil in 200 c.c. of water for five minutes were added and sufficient sterile water supplied to bring the moisture content up to the optimum. The tests were then incubated for 10 days, after which the sulfates were estimated as usual.

Table XI gives the arrangement of the experiment, together with the results secured. On examining these results, it appears that the smallest amount of gypsum had practically no effect on the sulfofying power of the soil, while the larger amounts depressed the production of sulfates. The greatest depression occurred with the use of 0.30 gm. of the sulfate, and when 0.50 gm. was added the depression was less but it was still greater than that with the 0.10 gm. of the sulfate.

The previous experiments which have shown the stimulating effect of gypsum were carried out in greenhouse soils and much smaller amounts were used than was the case here, so that these results are not in any way opposed to the earlier ones. It was apparent in those results that applications of gypsum at a rate sometimes employed in field soils stimulated sulfofication and hence it is evident that the application of gypsum cannot be increased to any appreciable extent without bringing about a depression in sulfofying power.



There could be no practical value, therefore, in making heavy applications of gypsum from the standpoint of bringing about an increase in sulfification. Of course, these results should not be accepted as conclusive for field practice because of the fact brought out in earlier work that gypsum is rather readily assimilated in the soil, hence there was probably some assimilation in these experiments and the results secured for the treated soils may have been too small. It was impossible to ascertain the extent of the assimilation and in making the calculations, the total amount of sulfate added was subtracted from the final figure.

TABLE XI.  
THE EFFECT OF  $\text{CaSO}_4$  ON SULFICATION.

No.	Treatment	Mg. S. as $\text{SO}_4$ after Incubation	Mg. S. as $\text{SO}_4$ added in $\text{CaSO}_4$	Mg.S.as $\text{SO}_4$ from free S.	Av. Per Cent S. Sulfified
1	Nothing .....	21.0	0	21.00	
2	Nothing .....	21.6	0	21.60	21.30
3	0.05 gm. $\text{CaSO}_4$ .....	28.4	9.35	19.05	
4	0.05 gm. $\text{CaSO}_4$ .....	30.6	9.35	21.25	20.15
5	0.075 gm. $\text{CaSO}_4$ .....	33.0	14.02	18.98	
6	0.075 gm. $\text{CaSO}_4$ .....	32.0	14.02	17.98	18.48
7	0.10 gm. $\text{CaSO}_4$ .....	37.6	18.69	18.91	
8	0.10 gm. $\text{CaSO}_4$ .....	36.4	18.69	17.71	18.31
9	0.30 gm. $\text{CaSO}_4$ .....	62.8	56.07	6.73	
10	0.30 gm. $\text{CaSO}_4$ .....	60.0	56.07	3.93	5.33
11	0.50 gm. $\text{CaSO}_4$ .....	106.0	93.45	12.55	
12	0.50 gm. $\text{CaSO}_4$ .....	110.0	93.45	16.55	14.55

It is safe to conclude, however, that the applications of gypsum which will give the most practically economic effect are those commonly employed in field practice.

### SERIES III.

#### THE EFFECT OF CALCIUM CARBONATE ON SULFICATION.

If sulfification is an important process occurring in field soils as seems to be the case, the effect of applications of calcium carbonate on its occurrence must be considered. Is it increased as are ammonification and nitrification, or it is decreased when the acidity of a soil is remedied by the use of limestone? This test was planned to throw some light on this point.

The same soil used in the preceding test was employed here. The soil was weighed out, the calcium carbonate in special amounts, and the sulfur added and stirred in, 10 c.c. of a fresh soil infusion applied, the moisture content adjusted to the optimum and the tests incubated for 10 days. The results of the sulfate determinations appear in Table XII. It appears clearly in this table that the use of calcium carbonate on an acid soil increased sulfification. There was a considerable increase when the acid-

ity of the soil, the lime requirement of which was 1072 pounds per acre of 2,000,000 pounds of surface soil, was neutralized and with further additions of calcium carbonate still greater gains in sulfofication were found. The greatest gain, however, occurred with the use of 0.3 gm. per 100 gm. of soil, corresponding to 6000 pounds to the acre, and beyond that point the increases were somewhat less.

TABLE XII.  
THE EFFECT OF  $\text{CaCO}_3$  ON SULFOFICATION

No.	Treatment	Mg. S. as $\text{SO}_4$ after Incubation	Average for Treatment
1	Nothing .....	21.0	
2	Nothing .....	21.9	21.4
3	Neutralized .....	27.2	
4	Neutralized .....	25.6	26.4
5	Neutralized plus 0.1 gm. $\text{CaCO}_3$ .....	32.2	
6	Neutralized plus 0.1 gm. $\text{CaCO}_3$ .....	32.8	32.5
7	Neutralized plus 0.3 gm. $\text{CaCO}_3$ .....	40.6	
8	Neutralized plus 0.3 gm. $\text{CaCO}_3$ .....	36.4	38.5
9	Neutralized plus 0.5 gm. $\text{CaCO}_3$ .....	32.6	
10	Neutralized plus 0.5 gm. $\text{CaCO}_3$ .....	33.4	33.0
11	Neutralized plus 1.0 gm. $\text{CaCO}_3$ .....	35.0	
12	Neutralized plus 1.0 gm. $\text{CaCO}_3$ .....	33.4	34.2
13	Neutralized plus 5.0 gm. $\text{CaCO}_3$ .....	32.6	
14	Neutralized plus 5.0 gm. $\text{CaCO}_3$ .....	30.4	31.5

If the applications of the carbonate had been increased still further, it is possible that the sulfofication would have decreased below that of the soil with its acidity just neutralized or even below that of the acid soil, but the amounts used here were not sufficiently large to bring about such a decrease. Inasmuch as the applications made in the field never exceed the amounts used here, there need be no apprehension of decreasing sulfofication by the use of ordinary amounts of calcium carbonate to remedy acid conditions in the soil.

On the other hand, it is evident from these results that calcium carbonate up to 6000 pounds per acre increased the sulfofying power of this soil. Larger amounts of the carbonate such as are rarely used in practice gave considerable increase in sulfofication, but these were somewhat less than those secured with the 3-ton amount.

#### SERIES IV.

##### THE EFFECT OF MAGNESIUM CARBONATE ON SULFOFICATION.

Having ascertained that calcium carbonate exerted a beneficial effect on sulfofication, it was deemed desirable to determine whether magnesium carbonate would have the same effect or not. This experiment was planned to throw some light on the question.

The soil used was the same as in the previous series. The arrangement of the test was the same as in the previous case except that no acid soil

On turning to Table XIII which gives the results of the tests, it is found that the smallest amount of magnesium carbonate increased slightly the sulfofying power of the soil, but the larger amounts gave gradually increasing depressions up to the largest amount employed here. It is evident that applications of magnesium carbonate in amounts greater than 2000 pounds per acre depressed the sulfofying power of this soil below that shown by the sample receiving no magnesium carbonate at all.

No.	Treatment	Mg. S. as SO <sub>4</sub> after Incubation	Average for Treatment
1	Nothing ..	24.4	
2	Nothing .....	25.7	25.0
3	0.1 gm. MgCO <sub>3</sub> .....	28.0	
4	0.1 gm. MgCO <sub>3</sub> .....	31.4	29.7
5	0.3 gm. MgCO <sub>3</sub> .....	26.5	
6	0.3 gm. MgCO <sub>3</sub> .....	21.4	23.9
7	0.5 gm. MgCO <sub>3</sub> .....	19.6	
8	0.5 gm. MgCO <sub>3</sub> .....	17.1	18.3
9	1.0 gm. MgCO <sub>3</sub> .....	17.0	
10	1.0 gm. MgCO <sub>3</sub> .....	16.5	16.7
11	5.0 gm. MgCO <sub>3</sub> .....	15.8	
12	5.0 gm. MgCO <sub>3</sub> .....	15.1	15.4

Evidently the sulfolyzing bacteria are much less sensitive to the presence of an abundance of calcium carbonate than to the presence of much magnesium carbonate. This is in accord with other bacteriological results dealing with the transformation of soil nitrogen, and it is in accord also with many crop results.

## SERIES V.

## THE EFFECT OF CALCIUM AND MAGNESIUM CARBONATES ON SULFOFICATION.

This test was planned to determine the effect of calcium and magnesium carbonates combined on sulfofication. The same soil and the same arrangement of the experiment was used here as in the two previous tests, except that both calcium and magnesium carbonates were applied. The amounts of these materials combined were the same as the amounts of the single substances used in the earlier series.

The results of the tests appear in Table XIV, and an examination of this table shows that the use of calcium and magnesium carbonates in amounts larger than 2000 pounds per acre of both together depressed the sulfofying power of this soil below that of the neutral soil. The check soils here represented the soil with its entire acidity neutralized with calcium carbonate. Increasing the application of calcium and magnesium carbonates together beyond 6000 pounds per acre decreased the sulfofying power of this soil, the depression increasing with increasing amounts applied.

TABLE XIV.

THE EFFECT OF  $\text{CaCO}_3$  PLUS  $\text{MgCO}_3$  ON SULFOFICATION.

No.	Treatment	Mg. S. as $\text{SO}_4$ after Incubation	Average for Treatment
1	Nothing .....	27.0	
2	Nothing .....	24.8	25.9
3	0.05 gm. $\text{CaCO}_3$ plus 0.05 gm. $\text{MgCO}_3$ .....	28.0	
4	0.05 gm. $\text{CaCO}_3$ plus 0.05 gm. $\text{MgCO}_3$ .....	20.0	29.0
5	0.15 gm. $\text{CaCO}_3$ plus 0.15 gm. $\text{MgCO}_3$ .....	25.7	
6	0.15 gm. $\text{CaCO}_3$ plus 0.15 gm. $\text{MgCO}_3$ .....	24.1	24.9
7	0.25 gm. $\text{CaCO}_3$ plus 0.25 gm. $\text{MgCO}_3$ .....	18.2	
8	0.25 gm. $\text{CaCO}_3$ plus 0.25 gm. $\text{MgCO}_3$ .....	21.4	19.8
9	0.50 gm. $\text{CaCO}_3$ plus 0.50 gm. $\text{MgCO}_3$ .....	16.1	
10	0.50 gm. $\text{CaCO}_3$ plus 0.50 gm. $\text{MgCO}_3$ .....	16.4	16.2
11	2.50 gm. $\text{CaCO}_3$ plus 2.50 gm. $\text{MgCO}_3$ .....	19.8	
12	2.50 gm. $\text{CaCO}_3$ plus 2.50 gm. $\text{MgCO}_3$ .....	22.3	21.0

It is apparent, therefore, that on this soil applications of calcium carbonate produced greater effects on sulfofication than the use of magnesium carbonate or combinations of the two carbonates. It is further evident the use of magnesium or dolomitic limestones on this soil, after its acidity has been neutralized with calcium carbonate may lead to a depression in sulfofying power, if the amounts used exceed 2000 pounds to the acre. On the other hand, non-magnesian limestones up to 6000 pounds per acre increased the sulfofying power of the soil, and in larger applications, produced smaller effects on sulfofication, but no actual depressions.

## CONCLUSIONS.

These tests lead to the following conclusions:

1. In the use of the free-sulfur-fresh-soil method for testing the sulfofying power of soils, the incubation period should be 14 days at room temperature to give the most conclusive results. Ten day's incubation gave the relative sulfofying powers of soils quite accurately, but the differences were much more distinctive for the longer period.

2. Calcium sulfate in ordinary applications had no detrimental effect on sulfofication, but very large applications might decrease the rate of oxidation of sulfur.

3. Calcium carbonate in ordinary applications on acid soils, increased sulfofication considerably and even in excessive amounts affected sulfur oxidation favorably.

4. Magnesium carbonate in small amounts increased sulfofication, but in large amounts depressed it even below that in the same soil with its acidity unneutralized.

5. Magnesium carbonate and calcium carbonate in combination exerted a beneficial influence on sulfofication when used in small amounts. Larger applications, however, depressed the oxidation of sulfur. The effects of the combined material were less than those of the calcium carbonate alone.

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# BACTERIAL NUMBERS IN SOILS, AT DIFFERENT DEPTHS, AND IN DIFFERENT SEASONS OF THE YEAR.<sup>1</sup>

By

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The latter half of the nineteenth century and the beginning of the twentieth witnessed the rapid development of the study of the biology of the soil. A great deal of work has been done along the line of types and physiological activities of the soil bacteria. This paper is limited to the investigation of the bacterial numbers in soils under different treatment, at different depths, and in different seasons of the year.

## HISTORICAL.

The early investigators in the field of soil bacteriology were looking for pathogenic organisms. The importance of bacteria as factors in the fertility of the soil has been revealed as a result of these investigations. Birsh-Hirschfeld, having studied a few Dresden soils in 1874, came to the conclusion that wet soil is more favorable for the growth of micro-organisms than dry soil. Pasteur, Koch, Tomasi, Crudeli, Tyde and Nicolaier, from 1877 to 1886 were finding different pathogens in the soil, and only Fodor, Dehérain and a few others were studying the micro-organisms as to their function in the soil itself.

Koch (20) was the first to point out in 1881 the fact that the numbers of bacteria in the soil are large, and decrease with an increase in depth. At a depth of about one meter the soil is almost free from bacteria. Proskauer (23) took his samples under absolutely sterile conditions and proved that the bacterial numbers decrease with depth. Beumer (1) was the first to dilute the soil before pouring his plate. He also found a great decrease with depth. Fränkel (13) made the first exact study of bacterial numbers in the soil. He was the first investigator to study virgin soils, not contaminated with sewage. He found a gradual decrease of bacterial numbers with depth of soil, from 90,000-300,000 at the surface, to 100-700 at a depth of 2.5 meters. The change in numbers with depth was not gradual, but sudden and irregular. He found larger numbers in summer than in winter, more in cultivated than in uncultivated soils. He concluded that the season of the year and surface covering have no great influence upon bacterial numbers at different depths.

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<sup>1</sup> Received for publication March 10, 1916.

Maggiora (22) found a decrease from 69,000,000 bacteria per gram of soil at the surface to 17,000,000 at a depth of 4 meters, and from 32,000,000 at the surface to 18,000 at a depth of 3 meters, but his large numbers are probably due to the fact that he allowed the soil to stand in the laboratory for a few days before examining it. Reimers (24) found a decrease from 2,564,000 organisms at the surface to none at a depth of 6 feet in stoney soil, and from 524,500 at the surface to 5,800 at a depth of 1.5 meters in wet meadow land. Houston (16) found a decrease from 1,680,000 bacteria per gram of soil at the surface to 410 at a depth of 6 feet. Caron (6), Stoklassa and Earnest (27), Kebrehl (18), Chester (7), King (19), Waite and Squires (29), and Brown (2) noted a decrease of bacterial numbers with depth. Chester demonstrated that soils rich in humus show larger numbers of bacteria than those low in humus. When, however, the quantity of humus in the soil is too high, deleterious products, especially those of an acid character, are produced which kill many bacteria and inhibit the development of others. Rich woodland is in this condition and always shows low numbers of bacteria. King (19) noted that the bacterial numbers commencing at the surface increase to a depth of 5 or 6 inches, depending upon the depth of plowing, and disappear at a depth of 7 feet below the surface. He concluded that the periods of maximum and minimum activity are, to a certain extent, independent of moisture and temperature and are possibly due to the presence of bacterial by-products. Brown (2) found the greatest number of organisms, in the different soil types, at a depth of 4 inches. The numbers decreased with depth, the greatest fall occurring within the first 12, and sometimes the first 8 inches.

As to the question of cultivation, most of the investigators seem to agree that soils of the same type contain larger numbers of bacteria when cultivated than when left uncultivated. This is shown by Houston (16), Fabricius and Feilitzen (11), Waite and Squires (29), and Burri (5). These investigators concluded that cultivation increases bacterial activities and available plant food. Fischer (12) found smaller bacterial numbers in a cultivated than in a non-cultivated moor soil. Engberding (10) found that the water content of the soil has an important bearing upon bacterial numbers; cultivation increases the numbers by increasing the water content.

Hiltner and Störmer (15) made a thorough study of cultivated and uncultivated soils and came to the conclusion that with similar types of soil and the same treatment, at the same depth, a unique microflora is found at definite periods. Samples taken under the same conditions gave identical results and possessed similar bacteriological characters; while the soils differing in any respect had different bacteriological relations. However, they believed that cultivation does not increase the

bacterial numbers that are able to grow on gelatin, but rather causes a decrease. Temple (28) found that the addition of cow manure to the soil greatly increases the number of bacteria in the soil and that this increase continues over a considerable period. Conn (9), examining different soils for their different contents, found a difference between 78,000,000 and 4,000,000 per gram. He thought that these differences are only small when compared with the variations of the bacterial counts of milk or water; also that the high and low counts are associated with high and low moisture contents, respectively, rather than with differences in soil type.

Very little attention has been paid to the numbers of microorganisms in the soil during the different seasons of the year. Kossowitz (20) found in several soil samples that he took in the winter that there were smaller bacterial numbers than those found in the same place in the summer, but he does not tell whether or not the soil was frozen when the samples were taken. Remy (25), taking his soil samples all through the growing season, found that the bacterial numbers depend on the moisture content of the soil, but he took no samples while the soil was frozen. Hiltner and Störmer (15) took several soil samples through the winter, but they did not find any great difference in bacterial numbers in the winter and in the summer. Their results seem to indicate that the numbers depend on the moisture content of the soil. Engberding (9) found that the variation of soil temperature had relatively little influence upon the bacterial numbers, which were rising and falling in the warm part of the year with the water content of the soil; long continued frost seemed to depress the numbers.

Conn (8), after a careful comparison of bacterial numbers in frozen and unfrozen soil, came to the conclusion that the number of bacteria in frozen soil is generally larger than in unfrozen soil, which is true not only of cropped soil, but also of sod and fallow land. This increase in bacterial numbers after freezing is not due to an increase in moisture content, even though in an unfrozen condition the bacterial numbers seemed to increase and decrease parallel to the moisture content of the soil. The increase in frozen soil seems to be due to an actual multiplication of the bacteria, rather than to a mere rise of the organisms from lower depths brought about by mechanical forces alone. Finally, there is the work of Brown and Smith (4), who confirm Conn's results of increased bacterial numbers in frozen soil. They advanced the theory that surface tension exerted by the soil particles on the films of water, the presence of salts in the water, and the concentration of the salts which may occur when the main body of water begins to freeze, all cause the hygroscopic water in soils to remain uncongealed, and consequently bacteria may live in it and multiply to a comparatively large extent.



Weber (30) also found that the action of low temperatures greatly increases the numbers of bacteria. Russell and Hutchinson (26) try to explain this phenomenon by the fact that the low temperatures suppress the protozoan activities in the soil, and for that reason allow the bacteria to multiply to large numbers. Given and Willis (14) obtained the lowest bacterial counts in the latter part of September, when the soil was very cold, but not frozen. Fairly high counts were obtained when the soil was frozen, but these were not the largest counts obtained through the year.

#### EXPERIMENTAL.

*Methods employed.* An effort was made to use a medium which would permit of the development of the largest possible number of bacteria. Several media commonly used for quantitative bacteriological work were compared. Brown's "egg-albumen" agar (3) was found to be the best medium for the development of the largest numbers of bacteria. This medium is composed as follows:

1000 c.c. water,  
10 gm. dextrose,  
.5 gm.  $K_2HPO_4$ ,  
.2 gm.  $MgSO_4$ ,  
.1 gm. egg-albumen,  
Trace of  $Fe_2(SO_4)_3$ ,  
15 gm. agar.

It was also found that .15 gm. of egg-albumen gave better results than .10 gm.; consequently, this medium was adopted in the following work. The egg-albumen was dissolved in a little cold water to which a few drops of NaOH were added, and this was added to the hot medium that was already prepared. If the albumen was dissolved first in a little NaOH it was found that no coagulation of the albumen took place, even upon mixing it with the hot medium. The latter was then tubed and sterilized at 10 pounds pressure for 30 minutes.

The plates were prepared by the usual dilution method. In order to avoid contamination, this procedure was followed: 200 c.c. Erlenmeyer flasks with cotton plugs were sterilized in a drying oven, cooled, and weighed. The soil in the sampling bottle was well mixed, and a portion of it was transferred with a sterilized spatula to the flask. The flask was weighed again and the weight of the transferred soil portion was determined by finding the difference. Sterile water was added to the flask in order to have a volume just ten times the weight of the sample. This gave a dilution of 1-10. The mixture was shaken for 5 minutes. Then the following dilutions were made, using 1 c.c. pipettes for the transfers: 1 c.c. of the infusion added to 199 c.c. of sterile water, gave a dilu-

tion 1-2,000; after shaking it well, 1 c.c. of this dilution was transferred into 99 c.c. of sterile water in the case of surface soil samples, where the bacterial numbers were large, so as to have a dilution 1-200,000. In the case of the subsoil samples 1 c.c. of the 2,000 dilution was transferred into 9 c.c. of sterile water, so as to have the dilution of 1-20,000. Plates were poured in triplicate from the highest dilutions and one plate from the lower dilution to serve as a check. In all cases the results of the highest dilutions made are given. The plates were incubated for six days at 20-22° C., at the end of which time the counts were made. The six-day period of incubation was compared with the three-day period, and since the longer period allowed a greater development of colonies, it was adopted.

*Methods of taking soil samples.* The instruments used were 24 cylindrical glass flasks, 10 inches high and 3 inches in diameter, plugged with cotton and sterilized; a small alcohol lamp, a knife, a ruler, a thermometer, a rag soaked in alcohol for sterilization of tools, a shovel, and a pickaxe. All precautions were taken to avoid any possible contamination. A pit approximately 12 inches wide, 30 inches long, and 30 to 32 inches deep was dug. With a sterilized knife the soil was removed at each depth prior to sampling. After removing the outside soil, the knife was again sterilized over the alcohol lamp, and the sample transferred to the sterilized bottle, after removal of the cotton plug. The plug was replaced at once, after the soil had been introduced into the bottle. When the soil was frozen, the soil had to be cut out with the pickaxe, which was used for making the pit; in that case the axe was carefully cleaned before sampling, with the rag soaked in alcohol.

The samples were taken to the laboratory, where the inoculations were made at once, uniformity being carefully obtained in making the infusion. In the case of frozen soil, a piece of it was introduced into a sterile flask, and regular treatment followed, the soil thawing out in the process of shaking. When the samples were brought into the laboratory, the bacterial inoculations were made first, and 100 gm. of soil from each sample were transferred into porcelain dishes. These were exposed to the air for a few days, and then weighed again, difference in weight being taken as the percentage of moisture in the original sample.

*Soils used.* Four soils were used, designated as A, B, C, and D. Soils A, B, and D are classed by the Bureau of Soils as Sassafraß loam, and C as Alloway clay loam. The mechanical composition of these soils is shown in the table on the following page.

Plot A is one of the plots of the Botanical Department of the New Jersey Agricultural Experiment Station. This plot has been manured for the last twenty years with 15 to 20 tons of stable manure per acre annually and has received an application of lime every five years. Gar-

den crops, such as peppers, tomatoes, beans, etc., have been grown on this plot every year without any regular rotation.

Plot B is the unfertilized plot of the apple orchard on the College Farm, at the New Jersey Station, near Plot A. The orchard was planted in 1896 for fertilizer experiments. Plot B is the check plot, which did not receive any fertilizer at all, but for the last three years oats have been grown as a cover crop. The orchard is cultivated during the summer from eight to ten times. The growth of the trees in the unfertilized plots is not any different from that of the plots receiving fertilizer every year. This shows that the soil is not poor in plant food, since it can compete for twenty years with fertilized plots in growing apple trees.

SASSAFRAS LOAM.<sup>1</sup>

	Organic Matter	Gravel 2-1 mm.	Coarse Sand 1-.5 mm.	Medium Sand .5-.25 mm.	Fine Sand .25-.1 mm.	Very Fine Sand 1-.05 mm.	Silt .05-.005 mm.	Clay .005-.0001 mm.
Surface .....	1.45	2.26	8.28	6.30	9.94	10.08	53.38	8.80
Subsoil .....	.98	1.48	5.80	5.64	10.56	11.34	49.16	15.70

ALLOWAY CLAY LOAM.

Surface .....	3.46	1.18	3.52	3.80	5.42	5.84	53.80	25.30
Subsoil .....	1.03	.80	3.96	4.98	6.88	7.10	46.82	28.76

Plot C is a timothy meadow which has been under grass for the last six years, and before that it was under oats and peas, and other forage crops.

Plot D is the wood-lot of the College Farm, which has not been plowed for at least 50 years, if at all.

In soils A, B, and C, in all instances, six samples were taken at depths of 1, 4, 8, 12, 20, and 30 inches, respectively. The first eight samples of Soil D were taken at all six depths, except in three cases, when, because of free water at the lowest depth, the sample was not taken at that point. The last five samples from Soil D were taken at depths of only 1 and 4 inches. A total of thirteen samplings were made, from January 30, 1915, to January 4, 1916.

Table I gives the climatic conditions through the year. The nitrogen content of the soil is found in Table II. This was determined by the Kjeldahl method. The total carbon determinations made by the official method are given in Table III. Table IV shows the lime-requirements of the soils determined by the Veitch method.

<sup>1</sup> This is taken from the "Soil Survey of the Trenton Area, in New Jersey," Bureau of Soils, 1902.

TABLE I.  
CLIMATIC CONDITIONS FOR THE YEAR 1915-1916.<sup>1</sup>

Date of Sampling	Air temperature at day of Sampling		Rainfall for the month Inches	Soil temperature			
	Maximum	Minimum		A	B	C	D
1915	° C.	° C.	° C.	° C.	° C.	° C.	° C.
Jan. 30.....	-2	-12	6.03	<sup>2</sup> -2	<sup>2</sup> -1	1.5	0.5
Feb. 12.....	9	2	5.81	<sup>2</sup> -1.5	<sup>2</sup> 0	2	1
Mar. 1.....	3	-6	.75	1	1.5	3	2.5
Mar. 23.....	18	1	.75	6	8	9	8
Apr. 16.....	20	0 5	2.75	10	10	12	11
May 8.....	25	18	3 87	12	11	14	13
June 3.....	16	8	2.66	16	14	20	19
July 7.....	27	12	5.96	22	20	21	23
Aug. 8.....	29.5	18	9 97	20	19	22	23
Sept. 10.....	30.5	21	2.06	24	23	24	21
Oct. 21.....	25	15	2.13	21	20	22	22
Nov. 30.....	6	-2	1 61	<sup>2</sup> 0	1	1	2
1916			December				
Jan. 4.....	10	-2	3.90	<sup>2</sup> -0 5	<sup>2</sup> 0	1	1

<sup>1</sup> The air temperature and rainfall statistics were taken from the records of the weather bureau stationed at the College Farm.

<sup>2</sup> At the date of sampling, the soil was frozen.

TABLE II.  
NITROGEN CONTENT, IN PER CENT, OF THE SOILS AT DIFFERENT DEPTHS.

Soil	1 inch	4 inches	8 inches	12 inches	20 inches	30 inches
A	0.1127	0.1176	0.0973	0.0581	0.0420	0.0581
B	0.1158	0.1071	0.1036	0.0819	0.0413	0.0287
C	0.1918	0.1596	0.1267	0.1050	0.0518	0.0350
D	0.2345	0.1015	0.0469	0.0294	0.0294	0.0307

TABLE III.  
CARBON CONTENT, IN PER CENT, OF THE SOILS AT DIFFERENT DEPTHS.

Soil	1 inch	4 inches	8 inches	12 inches	20 inches	30 inches
A	1.56	1.41	1.76	0.57	0.63	0.67
B	0.90	0.41	0.44	0.41	0.18	0.17
C	1.70	1.12	1.05	0.53	0.20	0.20
D	3.38	1.58	0.56	0.23	0.09	0.12

TABLE IV.  
LIME-REQUIREMENT, IN POUNDS, OF THE SOILS AT DIFFERENT DEPTHS.

Soil	1 inch	4 inches	8 inches	12 inches	20 inches	30 inches
A	200	100	0	0	0	0
B	2500	2000	1800	1600	800	1000
C	500	500	600	500	600	400
D	4600	3400	2400	2100	1800	1600

When the data presented in Table V are examined, one sees that in all cases the bacteria decreased with depth, except for the first four inches. In some cases the highest numbers were found at a depth of 1 inch from the surface, while in other cases the numbers increased from depths of 1 to 4 inches, then decreased regularly. On the average, the

TABLE V.  
NUMBER OF BACTERIA PER GRAM OF AIR-DRIED GARDEN SOIL—A.

Date of Sampling	1 inch	4 inches	8 inches	12 inches	20 inches	30 inches	Average
1915							
Jan. 30..	8,700,000	12,420,000	3,440,000	1,986,000	301,000	381,000	4,538,000
Feb. 12....	5,965,000	6,161,000	5,551,000	1,075,000	759,000	503,000	3,336,000
Mar. 1	8,812,000	5,531,000	3,370,000	1,591,000	1,183,000	849,000	3,556,000
Mar. 23	4,272,000	5,033,000	3,154,000	1,787,000	400,000	310,000	2,493,000
Apr. 16.	10,700,000	21,400,000	3,300,000	1,690,000	690,000	606,000	6,398,000
May 8...	4,760,000	8,410,000	4,150,000	1,050,000	554,000	200,000	3,187,000
June 3. .	6,890,000	5,090,000	4,445,000	2,420,000	2,100,000	410,000	3,559,000
July 7. .	7,760,000	6,220,000	2,810,000	800,000	310,000	300,000	3,033,000
Aug. 8	5,000,000	6,670,000	4,000,000	817,000	533,000	420,000	2,907,000
Sept. 10. .	8,767,000	7,100,000	4,150,000	1,217,000	353,000	127,000	3,619,000
Oct. 21. .	5,900,000	6,000,000	5,200,000	1,300,000	550,000	500,000	3,242,000
Nov. 30..	6,900,000	6,000,000	5,400,000	985,000	456,000	290,000	3,338,000
1916							
Jan. 4	9,200,000	4,540,000	3,000,000	340,000	220,000	56,000	2,893,000
Average .....	7,202,000	7,737,000	3,998,000	1,312,000	624,000	381,000	

highest bacterial numbers were found in the garden soil at a depth of 4 inches; the 1-inch depth gave a slightly smaller average. Below 4 inches the numbers decreased rapidly, the greatest fall occurring between depths of 4 and 8 inches. When the moisture content of the garden soil given in Table VI is compared with the bacterial numbers, one sees that

TABLE VI.  
MOISTURE CONTENT, IN PER CENT, OF GARDEN SOIL AT DIFFERENT DEPTHS.

Date of Sampling	1 inch	4 inches	8 inches	12 inches	20 inches	30 inches	Average
1915							
Jan. 30.....	15	12	9	6	7	9	9.67
Feb. 12.....	14	21	22	7	7	10	13.50
Mar. 1.....	13	11.8	8.7	7	7	12	9.92
Mar. 23.....	8	10	9	6	10	12	9.17
Apr. 16.....	9	10	11	8	7	12	9.50
May 8.....	9	12	10	11	11	10	10.50
June 3.....	9	11	10	9	8	11	9.67
July 7.....	9	10	10	7	10	9	9.17
Aug. 8.....	12	12	10	9	12	13	11.33
Sept. 10.....	10	11	9	9	10	11	10.00
Oct. 21.....	9	8	9	7	7	8	8.00
Nov. 30.....	9	9	9	8	10	11	9.33
1916							
Jan. 4.....	19	14	12	8	8	9	11.67
Average .....	11.15	11.68	10.67	7.85	8.77	10.54	

also in the case of moisture, the content rose from the 1-inch depth to the 4-inch depth, then decreased below the depth of 4 inches. But at depths lower than 12 inches, where the moisture content began to increase, the bacterial numbers decreased regularly. This is easily understood, since the lower depths, probably because of the exclusion of air and plant food, do not favor bacterial development.

The highest bacterial numbers throughout the year were found in Soil A on April 16, when 21,700,000 bacteria were found at a depth of 4 inches, and 10,700,000 one inch from the surface. On January 30, February 12, November 30, and January 4, the soil was frozen to a depth of 6 or 8 inches. The bacterial numbers from the samples taken on those dates are fairly high, but not the highest. In regard to the relation between the bacterial numbers and moisture content through the different seasons of the year, there does not seem to be any close association in the data presented in Tables V and VI.

The bacterial numbers and moisture content of Soil B are given in Table VII and VIII. This soil, which contains a much smaller amount of organic matter than Soil A, as seen from Table III, is as rich in nitrogen as the other soil, giving a narrower carbon-nitrogen ratio than Soil A.

TABLE VII.  
NUMBER OF BACTERIA PER GRAM OF AIR DRIED ORCHARD SOIL—B.

Date of Sampling	1 inch	4 inches	8 inches	12 inches	20 inches	30 inches	Average
1915							
Jan. 30.....	15,430,000	3,590,000	3,000,000	2,202,000	844,000	277,000	4,224,000
Feb. 12.....	6,824,000	4,852,000	3,370,000	1,123,000	1,036,000	468,000	2,945,000
Mar. 1.....	10,422,000	6,897,000	6,519,000	4,556,000	1,068,000	525,000	4,998,000
Mar. 23.....	4,538,000	5,622,000	2,101,000	1,615,000	463,000	753,000	2,515,000
Apr. 16.....	8,250,000	6,170,000	2,030,000	1,650,000	386,000	347,000	3,139,000
May 8.....	6,810,000	3,790,000	2,550,000	1,020,000	990,000	322,000	2,580,000
June 3.....	17,700,000	9,480,000	5,556,000	1,660,000	281,000	210,000	5,814,000
July 7.....	6,230,000	3,700,000	1,010,000	815,000	75,000	52,000	1,960,000
Aug. 8.....	4,833,000	5,000,000	1,867,000	810,000	367,000	340,000	2,203,000
Sept. 10.....	7,600,000	6,900,000	3,250,000	1,500,000	156,000	60,000	3,244,000
Oct. 21.....	5,800,000	6,500,000	2,270,000	1,270,000	720,000	560,000	2,853,000
Nov. 30.....	5,800,000	4,350,000	2,500,000	1,010,000	620,000	540,000	2,470,000
1916							
Jan. 4.....	7,100,000	5,650,000	1,200,000	620,000	110,000	60,000	2,457,000
Average .....	8,257,000	5,577,000	2,863,000	1,527,000	547,000	347,000	

The lime-requirement of Soil B is also higher than that of A. When the bacterial numbers are compared, one finds in Soil B the highest numbers just below the surface. This is probably due to the fact that the land is always shaded and the first inch of soil is not so dry as that of Soil A. The bacterial numbers decrease rapidly and regularly with depth, the greatest fall occurring between the depths of 1 to 4 and 4 to 8 inches. The highest bacterial numbers for this soil were found June 7. Also in

Soil B the frozen samples contained fairly large numbers of bacteria, but not the highest throughout the year.

TABLE VIII.  
MOISTURE CONTENT, IN PER CENT, OF ORCHARD SOIL AT DIFFERENT DEPTHS.

Date of Sampling	1 inch	4 inches	8 inches	12 inches	20 inches	30 inches	Average
1915							
Jan. 30.....	14	11	11	10	6	11	10.50
Feb. 12.....	15	19	19	11	8	9	13.50
Mar. 1.....	12.6	13	10	10	7	10	10.20
Mar. 23.....	9	10	11	9	5	8	8.67
Apr. 16.....	9.5	10.5	11.5	11	5	8	8.58
May 8.....	9	12	11	8	8	9	9.50
June 3.....	11	10	11	10	11	12	10.83
July 7.....	9	10	11	10	9	9	9.67
Aug. 8.....	11	11	10	8	9	10	9.83
Sept. 10.....	8	10	10	7	5	8	8.00
Oct. 21.....	9	10	9	7	5	6	7.67
Nov. 30.....	10	11	11	11	8	9	10.00
1916							
Jan. 4.....	22	15	14	8	11	11	13.50
Average .....	11.47	11.73	11.50	9.23	7.46	9.23	

It is seen from Table I° that the bacterial numbers in Soil C vary with depth in a manner similar to those in Soil B, the largest numbers occurring at a depth of 1 inch, the numbers then decreasing rapidly with depth. This soil gave, on the average, higher bacterial numbers than the other two

TABLE IX.  
NUMBER OF BACTERIA PER GRAM OF AIR-DRIED MEADOW SOIL—C.

Date of Sampling	1 inch	4 inches	8 inches	12 inches	20 inches	30 inches	Average
1915							
Jan. 30.....	6,267,000	2,325,000	1,609,000	556,000	206,000	198,000	1,860,000
Feb. 12.....	9,577,000	2,566,000	2,588,000	1,008,000	427,000	531,000	2,783,000
Mar. 1.....	9,478,000	5,926,000	3,176,000	1,132,000	232,000	162,000	3,351,000
Mar. 23.....	8,607,000	6,904,000	5,259,000	1,682,000	172,000	167,000	3,798,000
Apr. 16.....	11,940,000	4,150,000	2,680,000	1,090,000	220,000	172,000	3,375,000
May 8.....	11,640,000	8,190,000	1,220,000	1,110,000	1,100,000	704,000	3,994,000
June 3.....	5,990,000	4,850,000	1,580,000	823,000	300,000	235,000	2,296,000
July 7.....	6,340,000	5,200,000	3,800,000	1,106,000	100,000	70,000	2,770,000
Aug. 8.....	9,417,000	5,200,000	4,367,000	617,000	237,000	89,000	3,321,000
Sept. 10.....	10,120,000	8,500,000	4,400,000	850,000	356,000	170,000	4,066,000
Oct. 21.....	19,250,000	9,420,000	2,500,000	1,520,000	1,250,000	400,000	5,723,000
Nov. 30.....	10,500,000	6,500,000	2,670,000	980,000	100,000	110,000	3,477,000
1916							
Jan. 4.....	12,600,000	5,120,000	1,200,000	620,000	110,000	60,000	3,285,000
Average .....	10,133,000	5,758,000	2,850,000	1,007,000	370,000	236,000	

soils, probably because of the higher nitrogen and organic matter content, and high moisture content, connected with moderate acidity. The largest bacterial numbers were found in this soil October 21. At no

period of sampling has this soil been frozen more than 1 inch deep, because of the heavy sod covering the ground, so that the conditions of soil freezing cannot be taken into account.

TABLE X.

MOISTURE CONTENT, IN PER CENT, OF MEADOW SOIL AT DIFFERENT DEPTHS.

Date of Sampling	1 inch	4 inches	8 inches	12 inches	20 inches	30 inches	Average
1915							
Jan. 30.....	25	17	13	16	16	9	16.00
Feb. 12.....	29	17	15	12	11	12	16.00
Mar. 1.....	17	19	19	16.6	17	18	17.77
Mar. 23.....	21	17	15	15	11	12	15.17
Apr. 16.....	19	15	13	14	17	15	15.50
May 8.....	13	13	13	13	16	13	13.50
June 3.....	11	12	12	11	14	15	12.50
July 7.....	17	14	12	12	13	14	13.67
Aug. 8.....	20	14	14	14	12	17	15.17
Sept. 10.....	15	14	13	11	11	12	12.67
Oct. 21.....	19	14	13	12	10	11	13.17
Nov. 30.....	18	19	11	11	16	17	15.33
1916							
Jan. 4.....	29	20	16	13	16	17	18.50
Average .....	19.46	15.77	13.77	13.12	13.85	14.00	

Soil D, though high in organic matter and nitrogen, especially in the upper four inches of soil, contains small numbers of bacteria. This is probably to be looked for in the high acid content of the soil and the undecomposed condition of its organic matter. As is shown elsewhere, this soil contains large numbers of fungi, and the fungus flora is more extensive than the bacterial flora. This is in accord with other investiga-

TABLE XI

BACTERIA PER GRAM OF AIR DRIED FOREST SOIL.—D.

Date of Sampling	1 inch	4 inches	8 inches	12 inches	20 inches	30 inches
1915						
Jan. 30... ..	2,778,000	1,058,000	609,000	429,000	156,000	.....
Feb. 12. ....	1,847,000	1,658,000	542,000	486,000	230,000	150,000
Mar. 1.....	3,219,000	701,000	338,000	361,000	139,000	.....
Mar. 23.....	2,155,000	805,000	468,000	144,000	232,000	.....
Apr. 16. ....	550,000	470,000	500,000	330,000	133,000	116,000
May 8. ....	2,120,000	1,940,000	667,000	445,000	345,000	156,000
June 3.....	734,000	785,000	459,000	237,000	80,000	74,000
July 7.....	1,004,000	340,000	270,000	60,000	40,000	23,000
Aug. 8.....	4,233,000	1,820,000	.....	.....	.....	.....
Sept. 10.....	1,800,000	1,120,000	.....	.....	.....	.....
Oct. 21.....	900,000	890,000	.....	.....	.....	.....
Nov. 30.....	2,640,000	1,780,000	.....	.....	.....	.....
1916						
Jan. 4.....	3,170,000	1,870,000	.....	.....	.....	.....
Average .....	2,088,000	1,172,000	482,000	311,000	169,000	104,000



tions, that moor, forest, and other acid soils, with a high amount of undecomposed organic matter, are poor in bacterial numbers, but contain a rich fungus flora.

TABLE XII.  
MOISTURE CONTENT, IN PER CENT, OF FOREST SOIL AT DIFFERENT DEPTHS.

Date of Sampling	1 in.	4 in.	Avg.	8 in.	12 in.	20 in.	30 in.	Avg.
1915								
Jan. 30.....	28	18	23.0	14	15	14	15	17.33
Feb. 12.....	28	24	26.0	17	15	16	20	20.00
Mar. 1.....	42	23	32.5	15	15	23	..	23.60
Mar. 23.....	29	23	26.0	13	12	11	16	17.33
Apr. 16.....	27	15	21.0	13	12	15	14	16.00
May 8.....	25	16	20.5	12	11	15	15	15.67
June 3.....	22	15	18.5	11	11	13	14	14.33
July 7.....	25	20	22.5	11	13	11	12	15.33
Aug. 8.....	24	19	21.5	..	..	..	..	.....
Sept. 10.....	23	11	17.0	..	..	..	..	.....
Oct. 21.....	18	13	15.5	..	..	..	..	.....
Nov. 30.....	30	22	26.0	..	..	..	..	.....
1916								
Jan. 4.....	37	26	31.5	..	..	..	..	.....
Average .....	27.54	18.85		13.25	13.00	14.75	15.14	

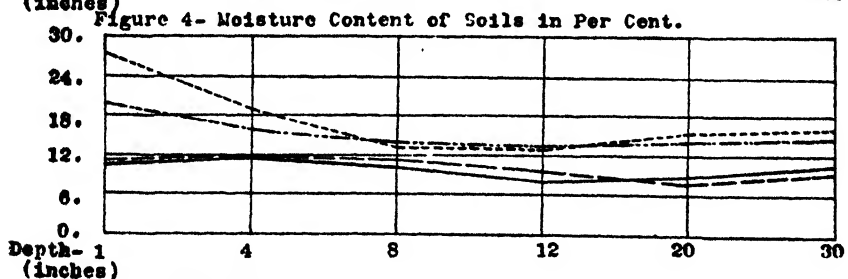
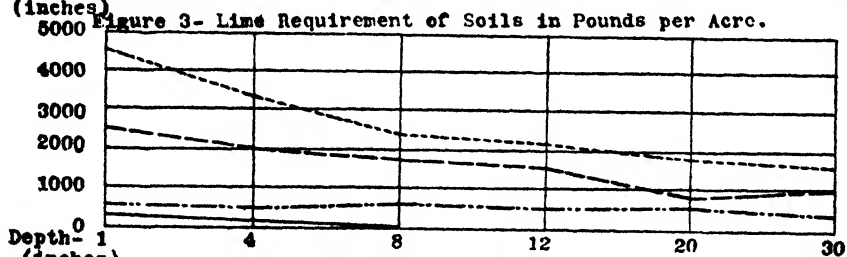
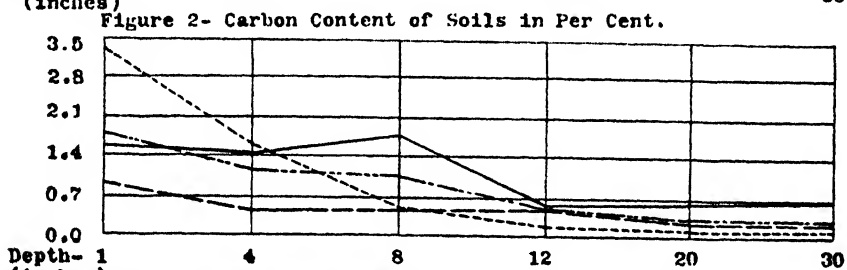
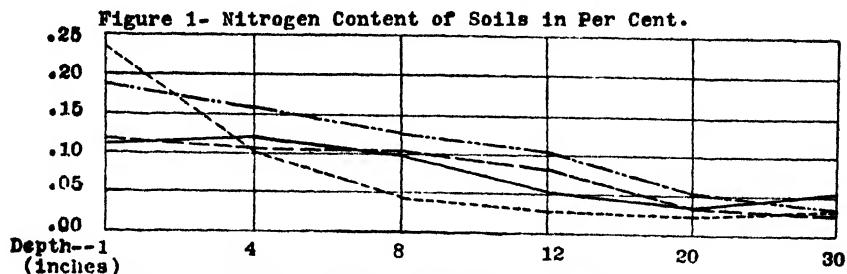
Considering now the results obtained from all the four soils, one finds that the conditions are quite uniform in relation to depth. Soil A, receiving large applications of stable manure and lime, has almost a neutral reaction, has its highest nitrogen and organic matter content at depths of

TABLE XIII.  
GENERAL AVERAGES FOR BACTERIAL NUMBERS IN RELATION TO DEPTH.

Soil	1 inch	4 inches	8 inches	12 inches	20 inches	30 inches
A	7,202,000	7,737,000	3,998,000	1,312,000	624,000	381,000
B	8,257,000	5,577,000	2,863,000	1,527,000	547,000	347,000
C	10,133,000	5,758,000	2,850,000	1,007,000	370,000	236,000
D	2,088,000	1,172,000	482,000	311,000	169,000	104,000

4 and 8 inches respectively (the fact that the highest organic content is at a depth of 8 inches is probably due to the fact that decomposition does not go on so rapidly at that depth as in the upper layer), and has the highest bacterial counts 4 inches from the surface. The other three soils, which received no application of manure, and were shaded most of the time, have the highest nitrogen, organic matter, and bacterial contents at a depth of 1 inch, or just below the surface, the numbers regularly decreasing with depth. This shows that not only the soil type and fertilization, but also the crops used have an important bearing upon the bacterial numbers. As to the lime requirements at the different soil depths, the highest lime requirement was found in all soils to be just below the sur-

face, the acidity decreasing regularly with the depth, the meadow soil having an almost uniform lime-requirement from the surface down to a depth of 30 inches.



Figures 1-4:

— Soil A.  
 --- Soil B.  
 ..... Soil C.  
 -.-.-.- Soil D.

Fig. 1-4. Content of nitrogen, carbon, lime and moisture in the four types of soil used.

The moisture content of the soils is highest 4 inches from the surface in the garden and orchard soils, and 1 inch from the surface in the meadow and forest soils. The moisture content decreases with depth to 12 or 20 inches below the surface; then it begins to increase.

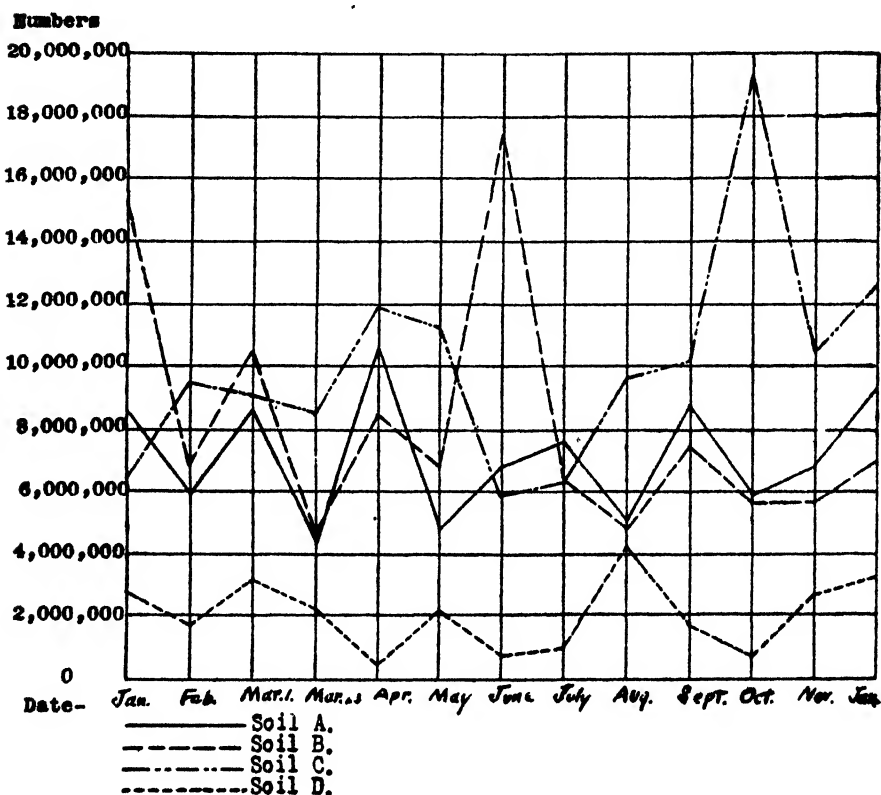


Fig. 5. Numbers of bacteria at the depth of 1 inch throughout the year.

The results, under the conditions at hand, with the culture media used, do not seem to confirm the conclusions of some investigators that the bacterial numbers are in direct relationship with the moisture content of the soil. The moisture, as well as the temperature, seem to have a bearing upon the numbers, but the changes in bacterial numbers cannot be explained by any of these conditions. For, in addition to the temperature and the moisture, there are so many influencing factors, such as soil type, soil treatment, crops used, condition of the organic matter of the soil, and soil reaction, that all of them have to be taken into consideration for the explanation of changes in the microörganic activities in the soil.

When one compares the changes in bacterial numbers in the different times of the year, he finds different results with the various soils used in this investigation. Soil A contained the highest numbers April 16, B—June 3, C—October 21, D—August 8. The frozen soil, though containing high bacterial counts, did not give the largest numbers found through the year. The variation of these results from those of Conn (7) and Brown (4) are to be looked for in the character of the soil, and the length of time during which the soils were frozen. As seen from the results of Brown's (4) investigation, the soil that he used was already

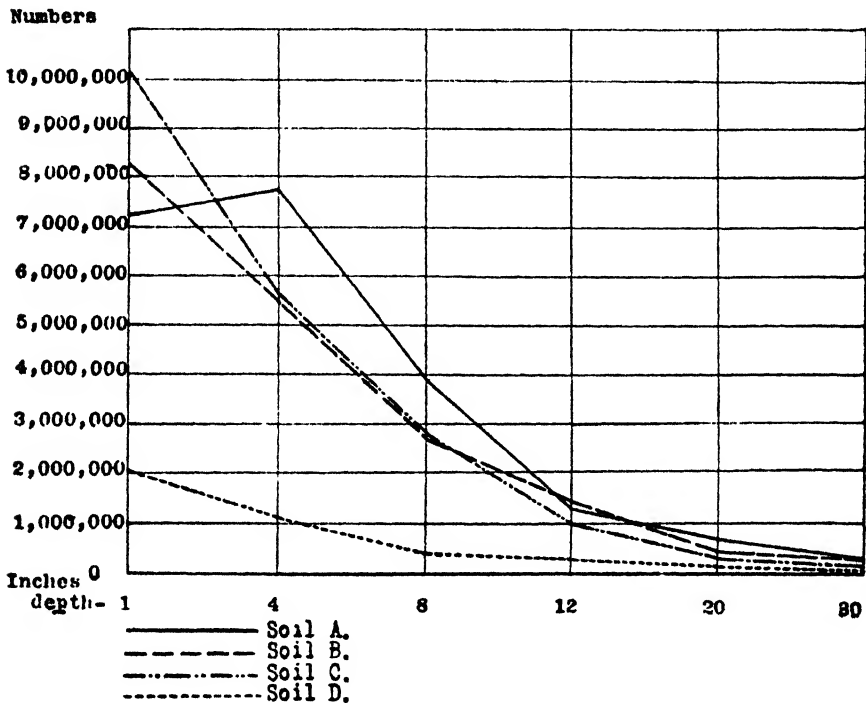


Fig. 6. Numbers of bacteria in different depths of soil: average for whole year.

frozen January 11, but he still found low bacterial numbers on that date, as well as on January 26 and February 11; and only on March 1, after the soil had been frozen almost two months, did he find an increase in bacterial numbers. The soils used in this experiment were at no time frozen continually for such a long period, and two of them (C and D) were never found to be frozen at all, when the samples were taken. The differences in the chemical and mechanical composition, soil type and treatment, and climatic conditions will probably account for the difference in the results.

## SUMMARY.

1. The greatest number of bacteria were found at a depth of 1 inch in the soils that are under shade all the year round. The garden (A) soil gave on the average the largest numbers 4 inches from the surface.
  2. There was a regular decrease in numbers of organisms from a depth of 1 inch (or 4 inches in the case of Soil A) down to a depth of 30 inches.
  3. The greatest decrease in numbers between any two consecutive depths of sampling occurred between the 1st and the 4th, or the 4th and the 8th inches.
  4. The meadow soil (C) gave the largest bacterial counts at a depth of 1 inch of all the soils, the 1-inch layer of this soil being richer also in organic matter and nitrogen content than that of soils A and B.
  5. The forest soil (D), though showing a high carbon and nitrogen content, gave the lowest bacterial counts probably because of the high acidity and large amount of undecomposed organic matter.
  6. The numbers of bacteria in the soils studied were not governed either by the moisture content of the different soils, or the nitrogen and carbon contents.
  7. There was a gradual decrease in the lime-requirement of the soils from the surface down to a depth of 30 inches, except in the meadow soil.
  8. There was also a more or less gradual decrease in the nitrogen and carbon content of the different soils from the surface down to a depth of 30 inches. As an exception, one finds Soil A, where the nitrogen content 4 inches below the surface was higher than at a depth of 1 inch. This is in accord with the increase in bacterial numbers and moisture content of that soil. Perhaps the moisture content, together with the humus and carbon content of the soil combined with its acidity, might account for the variations in bacterial numbers.
  9. Frozen soil, though showing a high bacterial content, did not give the largest bacterial numbers through the year. This may be due to the fact that the soils under study have never been frozen for a longer period than 8 or 10 days.
  10. The time of maximum bacterial numbers during the year varied with the different soils throughout the year; no two soils showed their maximum bacterial content at the time of any one sampling.
- In conclusion, the author wishes to express his sincere thanks to Dr. J. G. Lipman for the helpful suggestions in outlining this work, and to Mr. R. E. Curtis for the assistance in taking the samples.

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# THE INOCULATION AND INCUBATION OF SOIL FUNGI.

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The attention of soil biologists has recently been directed to a group of organisms, namely soil fungi, the activities of which may prove of no inconsiderable importance in the problems of soil fertility. It has been assumed from time to time, as for example in the protozoan theory advanced by Russell and Hutchinson (14) that ammonia production may be regarded as serving in some degree as an index to soil fertility. Undoubtedly this has likewise been the basis for what little experimentation has heretofore been recorded on the production of ammonia by soil fungi. Marchal (12), Müntz and Coudon (13), McLean and Wilson (11), Waksman and Cook (15) and Coleman (2), have presented data which point definitely to the fact that many soil fungi are capable of producing more ammonia from organic nitrogenous compounds than even the most efficient bacterial ammonifiers, such as those belonging to the *Mycoides* group. Presumably, the methods employed in soil bacteriology will be adapted, with the necessary modifications, to the study of the activities of soil fungi. However, a certain amount of preliminary data concerning fundamentals in methodology is prerequisite to further investigation in this branch of soil biology and it is in this spirit that the following experiments were planned.

In general, the procedure in ammonification studies with pure cultures of bacteria and fungi have been identical. It is unnecessary at this time to advance the evidence justifying the adoption of soil as a medium in the study of soil biology problems; suffice it to say that it has been so widely accepted as to warrant its use in this and similar experimentation. The organic nitrogenous materials most commonly employed are of animal and of vegetable origin, dried blood and cottonseed meal, respectively. The method followed by the most recent investigators regarding the inoculating materials, is to use a measured quantity of spores of the organism concerned.

<sup>1</sup> Part I of thesis submitted in partial fulfilment for the degree of M.Sc.

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## I. INOCULATION STUDIES WITH CERTAIN SOIL FUNGI.

## METHODS.

In the experiments to be considered presently, the inoculation material was prepared as follows: One-hundred cubic centimeter portions of Cook's No. II fungi medium which contains

Water .....	1000 c.c.
Glucose .....	20 gm.
Peptone .....	10 gm.
K <sub>2</sub> HPO <sub>4</sub> .....	0.25 gm.
MgSO <sub>4</sub> .....	0.25 gm.

were sterilized in 250-c.c. Erlenmeyer flasks and a few spores, from a pedigree culture of the fungus to be studied, introduced into the medium. It was then incubated at room temperature long enough to allow an abundance of spores to appear on the growth of mycelia which covered the surface of the liquid. The period of time necessary for such spore formation varies from 7 to 14 days, depending upon the individual organism. In order to get the spores distributed throughout the liquid, the flask is whirled for about ten minutes. In the case of *Penicillium* and *Zygorrhyncus* it was advisable to scrape the surface growth with a sterile platinum needle, in order to increase the number of spores which would otherwise be quite scanty. The liquid containing the spores in suspension was then transferred to a sterile flask for the purpose of leaving behind as much of the mycelia as possible, because of the fact that the latter clogs the pipette which is used subsequently to deliver the inoculum.

The soil used was Norfolk sandy loam having a lime requirement of 2,300 pounds CaO per acre, which is a favorable reaction for the activities of soil fungi, as reported in Part II of this thesis (8). One hundred fifty-five mg. N. in the form of dried blood and cottonseed meal, respectively, were added to 100-gm. portions of the soil, which were then made up to optimum moisture content by the addition of 13 c.c. of water and 3 c.c. for each gram of organic matter used. Proper deduction was made for the different amounts of inoculum added. The 200-c.c. cotton-plugged Erlenmeyer flasks containing soil were sterilized in the autoclave at 15 pounds pressure for 15 minutes. This process is, of course, responsible for the release of some additional available nutrients (1). After allowing the soil to become cool it was inoculated with the desired amount of spore-suspension by means of a sterile pipette. The necessary precautions against contamination must be strictly observed in such manipulation. The flask containing the inoculum was whirled thoroughly prior to each pipetting to ensure an even distribution of spores. Qualitative tests were carried out on Lipman and Brown's modified synthetic agar (10) for

bacterial contamination. The flasks containing soil were incubated for 7 days at 20° to 22° C., at the end of which time the soil was transferred to copper flasks, and ammonia distilled according to the magnesium oxide method, titrating with tenth normal acid and alkali.

The amounts of inoculum added in both series of organic matter were 0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 3.0, 4.0 and 5.0 c.c. respectively. In order to ascertain precisely the number of spores which were present in each of the units mentioned above, a count was made with each fungus for the number of spores per 1 c.c. of spore-suspension. The apparatus used for counting blood corpuscles (Blutkörperzählapparat) employed by Kopeloff, Lint and Coleman (9) in the counting of soil protozoa was adapted to this purpose. Since the fungus spores have, comparatively speaking, no motility, the experimental error of 5 per cent found previously would be somewhat reduced in the present determinations.

#### EXPERIMENTAL.

The fungi employed were isolated in pure pedigree culture from soil on the College Farm. Goddard (6), Jensen (7), and Dale (4, 5) make mention of some of these organisms, which with the assistance of Mr.

TABLE I.  
INOCULATION—PENICILLIUM SP. 10.

Lab No.	Spores c.c.	Organic Matter	H <sub>2</sub> O c.c.	Mg.N.	Mg. N.	Av.Mg.N.	Increase over ch'k Mg. N.	No. of Spores
131-132	0.2	155 Mg.N. Dried Bld.	16.5	6.70	6.80	6.75	3.45	19,200
133-134	0.4	"	16.3	8.83	9.31	9.07	5.77	38,400
135-136	0.6	"	16.1	14.82	16.21	15.52	12.22	57,600
137-138	0.8	"	15.9	15.30	12.90	14.10	10.80	76,800
139-140	1.0	"	15.7	15.92	15.92	15.92	12.62	96,000
141-142	2.0	"	14.7	21.51	21.51	21.51	18.21	192,000
143-144	3.0	"	13.7	24.70	22.50	23.60	20.30	288,000
145-146	4.0	"	12.7	26.98	27.42	27.20	23.90	384,000
147-148	5.0	"	11.7	27.84	26.37	27.21	23.91	480,000
149-150	0.2	155 Mg.N. Cot'nseed	20.3	9.78	10.61	10.20	6.40	19,200
151-152	0.4	Meal	20.1	12.73	12.28	12.51	8.71	38,400
153-154	0.6	"	19.9	12.19	13.60	12.90	9.10	57,600
155-156	0.8	"	19.7	16.86	16.74	16.80	13.00	76,800
157-158	1.0	"	19.5	21.18	21.83	21.51	17.71	96,000
159-160	2.0	"	18.5	24.86	24.03	24.45	20.65	192,000
161-162	3.0	"	17.5	28.91	29.00	28.96	25.16	288,000
163-164	4.0	"	16.5	25.80	26.60	26.20	22.40	384,000
165-166	5.0	"	15.5	33.00	32.01	32.51	28.71	480,000

S. A. Waksman were identified as follows: *Rhizopus Oryzae* (Wendt), *Zygorrhynchus Vuilleminii* (Namyslowski), *Rhizopus nigricans* (Ehrenberg), *Penicillium* sp. 10.<sup>1</sup> It should be noted that these identifications

<sup>1</sup> This organism appears to be identical with a member of Group 10 of soil *Penicillia* studied by S. A. Waksman, the description of which will appear at a later date.

are open to question, since time did not permit of corroboration by mycological specialists.

Considering first the effect of inoculating various quantities of spores of *Penicillium* sp. 10, as recorded in Table I and graphically illustrated in figure 1, it will be observed that there were present 96,000 spores per 1 c.c. of inoculum. Thus the range from 0.2 to 5.0 c.c. represents the gradation in numbers of spores from 19,200 to 480,000. It will be observed that in the dried blood series there is, with one exception, a gradual increase in ammonia accumulation with increasing quantities of inoculum

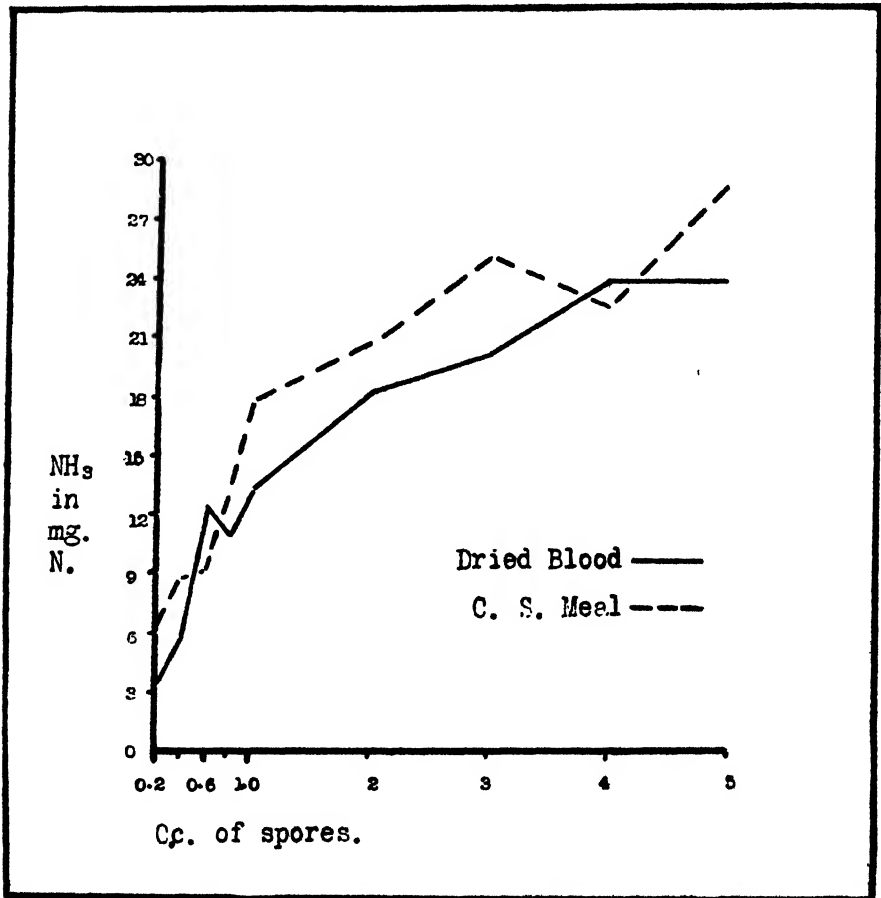


Fig. 1. Inoculation of *Penicillium* sp. 10. Increase over check of ammonia in mg. N. up to 4 c.c., which represents 384,000 spores. The increase is even more perceptible in the cottonseed meal series (with the exception of 4 c.c., where there is a depression for some unaccountable reason). Thus it

appears that with this organism the number of spores present directly influences the amount of ammonia accumulated. However, the increase in ammonia is not as great as the increase in spores, for where there is a 500 per cent increase of the latter, the corresponding increase in ammonia is in general about 150 per cent, or only one-third as great. There is, then, good reason to believe that only a limited number of spores become effective in influencing ammonia accumulation. That this is largely due

TABLE II.  
INOCULATION—RHIZOPUS NIGRICANS.

Lab. No.	Spores c.c.	Organic Matter	H <sub>2</sub> O c.c.	Mg. N.	Mg. N.	Av. Mg. N.	Increase over ch'k Mg. N.	No. of Spores
		155 Mg. N. Dried Bld.						
70-71	0.2		16.5	19 53	17.84	18 69	15.39	79,200
72-73	0.4	"	16.3	26.94	26.94	26.94	23.64	158,400
74-75	0.6	"	16.1	21 38	23.69	22.54	19.24	237,600
76-77	0.8	"	15.9	28 91	28.76	28.84	25.54	316,800
78-79	1.0	"	15.7	29 07	27.39	28.23	24.93	396,000
200-201	1.0	"	15.7	38 14	35.76	36.95	33.65	700,000
202-203	2.0	"	14.7	41 06	41.05	41 06	37.76	1,400,000
204-205	3.0	"	13.7	38 76	39.07	38.92	35.62	2,100,000
206-207	4.0	"	12.7	39 45	42.30	40.88	37.58	2,800,000
208-209	5.0	"	11.7	50 02	51.00	50.51	47.21	3,500,000
		155 Mg. N. Cot'nseed						
80-81	0.2	Meal	20.3	36 07	34.76	35.42	31.62	79,200
82-83	0.4	"	20.1	33 88	36.94	35.41	31.61	158,400
84-85	0.6	"	19.9	39.45	37.57	38.51	34.71	237,600
86-87	0.8	"	19.7	38 79	37.83	38.31	34.51	316,800
88-89	1.0	"	19.5	39.80	39.90	39.85	36.05	396,000
210-211	1.0	"	19.5	41 65	43.74	42.70	38.90	700,000
212-213	2.0	"	18.5	41 97	42.88	42.43	38.63	1,400,000
214-215	3.0	"	17.5	41 60	42.76	42.18	38.38	2,100,000
216-217	4.0	"	16.5	53.41	49.52	51.47	47.67	2,800,000
218-219	5.0	"	15.5	54.95	57.35	56.15	52.35	3,500,000

to a question of food supply is probable, since it is apparent that the conditions are hardly favorable for the complete utilization of the organic nitrogen present. Again, limited germination due to the operation of other factors might be responsible for the above-mentioned phenomenon. The accumulation of ammonia as measured in such experimentation represents the resultant of the two concomitant factors of production and consumption, consequently complexity must be anticipated.

From the data presented in Table II and its graphic representation in figure 2, it will be noted that the inoculation of spores of *Rhizopus nigricans* in increasing amounts effects, in a general way, an increase in the amount of ammonia accumulated. There are several exceptions to be noted in the dried blood series, while in the cottonseed meal series the gradations are not sharply defined. However, the tendency towards parallel increase is evident when one compares the ammonia produced

upon the inoculation of 0.2 c.c., representing 79,200 spores, and of 1.0 c.c., representing 396,000 spores. In the dried blood series, the former is 15.39 mg. of nitrogen compared with 24.93 mg. of nitrogen, or an increase of about 62 per cent; in the cottonseed meal series it is 31.62 as compared to 36.05 mg. of nitrogen, or an increase of about 14 per cent.

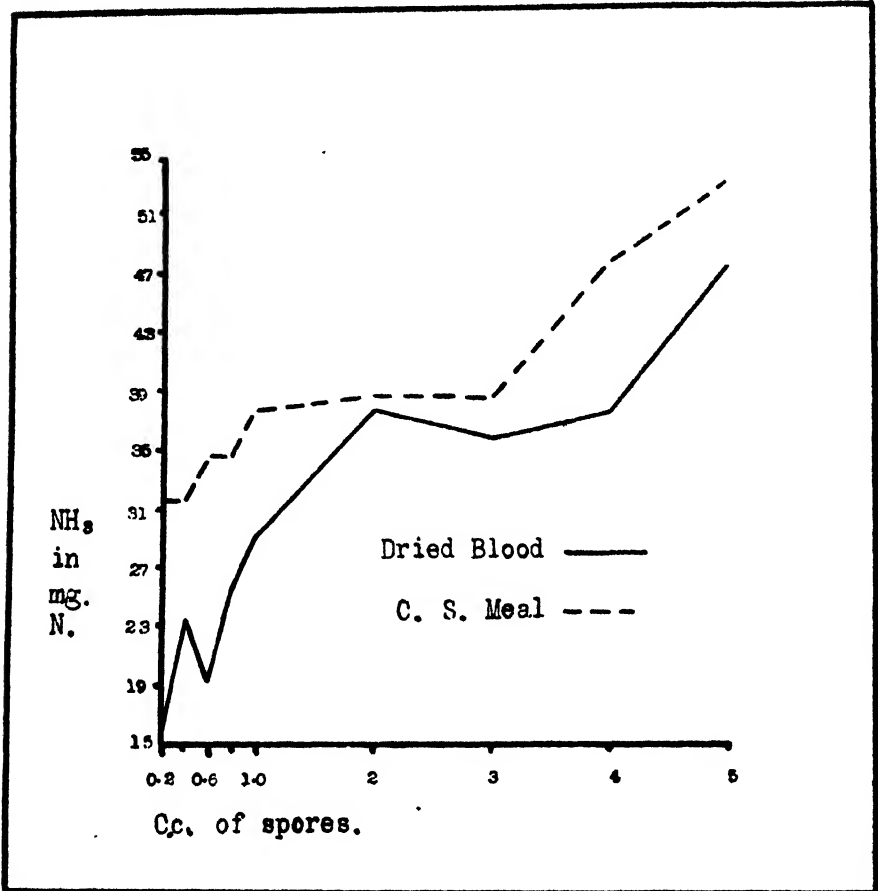


Fig. 2. Inoculation of *Rhizopus nigricans*. Increase in ammonia over check in mg. N.

Comparing the ammonia accumulated when 1 c.c. of inoculum representing 700,000 spores is used as against 5 c.c. or 3,500,000 spores, in the dried blood series there is an increase from 33.65 to 47.21 mg. of nitrogen, or about 40 per cent, whereas in the cottonseed meal series there is an increase from 38.90 to 52.35 mg. of nitrogen, or about 35 per cent.

It will be observed that two quantities of spores are recorded as present in 1 c.c. of inoculum. This is a result of having performed the inoculation of 0.2 to 1.0 c.c. at a different time from the inoculation of 1 to

5 c.c. However, this only seems to indicate more emphatically, perhaps, that the number of spores added has a direct influence on ammonia accumulation. For in the case of dried blood, the difference in ammonia between inoculation with 396,000 as compared with 700,000 spores is 8.8 mg. of nitrogen, or about 33 per cent increase. With cottonseed meal, the difference is comparatively insignificant, being only 3 mg. of nitrogen, or 8 per cent increase. Again, it appears that there is no evidence of a direct quantitative proportion obtaining between the increase in the number of spores added and an increase in ammonia accumulation. However, despite the lack of entire consistency in the results, there is a strong indication that with *Rhizopus nigricans* an increase in the number of spores added, is accompanied by an increase in ammonia accumulation with both of the organic nitrogenous materials employed.

TABLE III.  
INOCULATION—*ZYGORRHYNUS VUILLEMINII*.

Lab. No.	Spores c.c.	Organic Matter	H <sub>2</sub> O c.c.	Mg. N.	Mg. N	Av. Mg. N.	Increase over ch'k Mg. N.	No. of Spores
		155 Mg. N. Dried Bld						
90-91	0.2		16.5	9.23	8.92	9.08	5.78	15,200
92-93	0.4	"	16.3	9.38	9.38	9.38	6.08	30,400
94-95	0.6	"	16.1	8.92	8.92	8.92	5.62	45,600
96-97	0.8	"	15.9	9.23	9.38	9.31	6.01	60,800
98-99	1.0	"	15.7	11.68	10.30	10.99	7.69	76,000
220-221	1.0	"	15.7	9.23	9.54	9.39	6.09	100,000
222-223	2.0	"	14.7	10.32	10.23	10.28	6.98	200,000
224-225	3.0	"	13.7	10.46	10.61	10.54	7.24	300,000
226-227	4.0	"	12.7	10.77	10.36	10.57	7.27	400,000
228-229	5.0	"	11.7	11.84	11.84	11.84	8.54	500,000
		155 Mg. N. Cot'nseed						
		Meal						
100-101	0.2		20.3	27.68	26.22	26.95	23.15	15,200
102-103	0.4	"	20.1	28.61	28.15	28.38	24.58	30,400
104-105	0.6	"	19.9	27.84	27.53	27.69	23.89	45,600
106-107	0.8	"	19.7	28.30	28.19	28.25	24.45	60,800
108-109	1.0	"	19.5	28.45	28.15	28.30	24.50	76,000
230-231	1.0	"	19.5	31.99	31.68	31.84	28.04	100,000
232-233	2.0	"	18.5	33.68	32.91	33.30	29.50	200,000
234-235	3.0	"	17.5	35.68	35.53	35.61	31.81	300,000
236-237	4.0	"	16.5	35.99	35.68	35.84	32.04	400,000
238-239	5.0	"	15.5	54.37	53.80	54.09	50.29	500,000

From the results recorded in Table III and figure 3, it will be observed that *Zygorrhynchus Vuilleminii* is ordinarily a poor ammonifier of dried blood. Where 1 to 5 c.c. of inoculum were employed however, there is a distinct increase in ammonia accumulation, the lower amounts giving no such definite indication. The same phenomenon is to be noted in the cottonseed meal series. In the latter series it will be seen that when 1 c.c. of inoculum contained 76,000 spores there were 24.50 mg. of nitrogen, while the inoculation of 100,000 spores accounted for an increase

up to 28.04 mg. of nitrogen. The dried blood series furnishes an exception to this rule. This might be explained, together with the rather uncertain results obtained by inoculating 0.2 to 1.0 c.c., upon the basis that this organism is especially dependent on its food supply, and only a limited number of its spores become effective unless the environmental conditions are favorable.

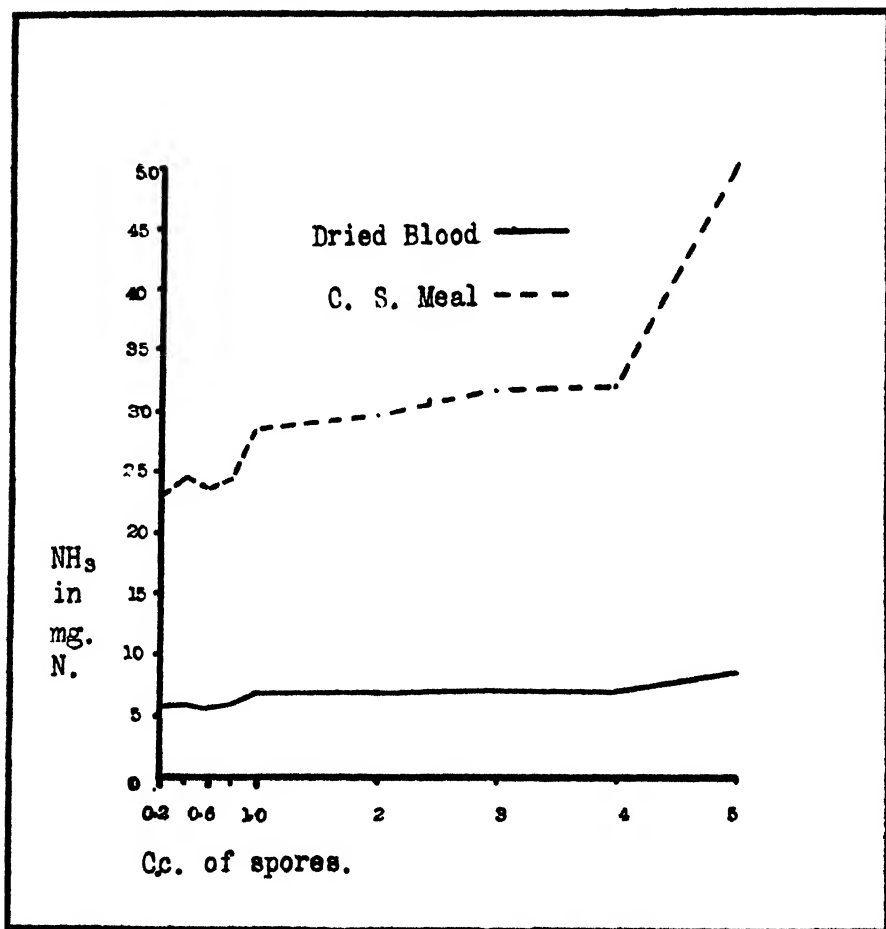


Fig. 3. Inoculation of *Zygorrhynchus Vuilleminii*. Increase over check of ammonia in mg. N.

Comparing the inoculation of 0.2 c.c. representing 15,200 spores with 1.0 c.c. representing 76,000 spores in the dried blood series, there is an increase of ammonia from 5.78 to 7.69 mg. of nitrogen, or about 33 per cent. In the cottonseed meal series there is a negligible increase of 6 per cent. In comparing the inoculation of 1 with 5 c.c. in the dried blood series, there is an increase in ammonia of 40 per cent, and in the cottonseed meal series of 79 per cent. This corroborates the evidence advanced

with *Penicillium* and *Rhizopus nigricans*, that an increase in the number of spores used for inoculation is accompanied by an increase in ammonia accumulation.

TABLE IV.  
INOCULATION—RHIZOPUS ORYZAE.

Lab. No.	Spores c.c.	Organic Matter	H <sub>2</sub> O c.c.	Mg. N.	Mg. N.	Av.Mg. N.	Increase over ch'k Mg. N.	No. of Spores
		155 Mg. N. Dried Bld.						
1-2	0.2		16.5	19.81	19.07	19.44	16.14	83,200
3-4	0.4	"	16.3	22.24	23.07	22.66	19.36	166,400
5-6	0.6	"	16.1	24.76	25.07	24.92	21.62	249,600
7-8	0.8	"	15.9	24.15	24.76	24.46	21.16	332,800
9-10	1.0	"	15.7	27.53	25.99	26.71	23.41	416,000
11-12	1.0	"	15.7	17.67	17.87	17.77	14.47	136,000
13-14	2.0	"	14.7	19.79	21.10	20.45	17.15	272,000
15-16	3.0	"	13.7	24.24	23.12	23.68	20.38	408,000
17-18	4.0	"	12.7	27.87	25.65	26.71	23.41	544,000
19-20	5.0	"	11.7	24.74	27.87	26.31	23.01	680,000
		155 Mg. N. Cot'n seed						
21-22	0.2	Meal	20.3	34.61	35.22	34.91	31.11	83,200
23-24	0.4	"	20.1	Lost	Lost	.....	.....	166,400
25-26	0.6	"	19.9	34.14	33.84	33.99	30.19	249,600
27-28	0.8	"	19.7	36.36	35.84	36.10	32.30	332,800
29-30	1.0	"	19.5	29.22	35.22	32.22	28.42	416,000
31-32	1.0	"	19.5	34.74	30.12	32.43	28.63	136,000
33-34	2.0	"	18.5	34.23	33.33	33.78	29.98	272,000
35-36	3.0	"	17.5	33.22	31.20	32.21	28.41	408,000
37-38	4.0	"	16.5	38.88	39.08	38.98	35.18	544,000
39-40	5.0	"	15.5	37.06	36.86	36.96	33.16	680,000

In discussing the results obtained with inoculation of *Rhizopus Oryzae* it is necessary to bear in mind that the inoculation of 0.2 to 1 c.c. was carried on at a different time from that of 1 to 5 c.c. In the latter case the spores per 1 c.c. were only one-third as great in number as in the former; thus the two series must be considered separately. Where dried blood was employed, there is a gradual increase (with one exception) in ammonia with an increase in inoculum from 0.2 to 1 c.c. The same is true in the series of inoculations with 1 to 5 c.c. Comparing the inoculation of 0.2 c.c. representing 83,200 spores with 1 c.c. representing 416,000 spores, there is an increase in ammonia from 16.14 to 23.41 mg. of nitrogen, or 45 per cent. Again, where 1 c.c. representing 136,000 spores is compared with 5 c.c. representing 680,000 spores, there is an increase in ammonia from 14.47 to 23.01 mg. of nitrogen, or 59 per cent. It is especially interesting to note that with 1 c.c. representing 136,000 spores there were 14.47 mg. of nitrogen, while with 416,000 spores in 1 c.c. there were 23.41 mg. of nitrogen, thus emphasizing again



the increase in ammonia accumulation with an increase in spores inoculated. The results of inoculation where cottonseed meal was used are too variable to allow of any definite conclusions.

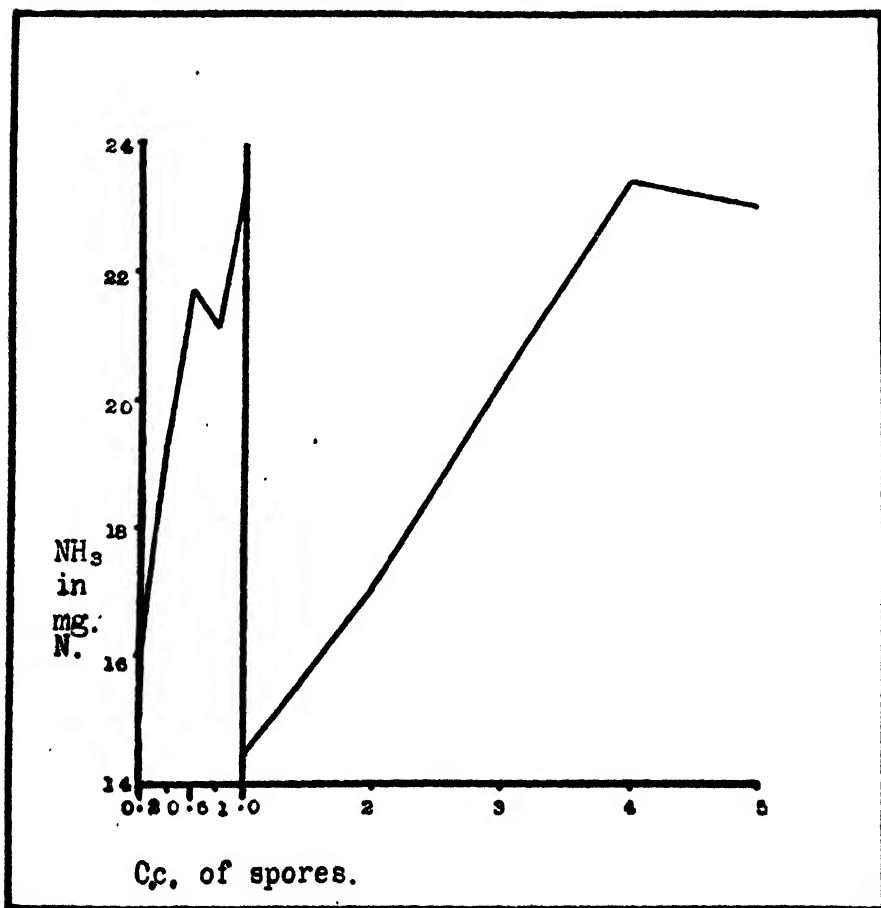


Fig. 4. Inoculation of *Rhizopus Oryzae* in dried blood. Increase over check of ammonia in mg. N.

The data bearing on the point of an increase in ammonia accumulation as a result of an increase in the number of spores inoculated is summarized in Table V. There are presented the ammonification results obtained while using dried blood and cottonseed meal, with 0.2, 1.0 and 5.0 c.c. of spores for inoculation. It may be remarked, parenthetically, that the results for 1 c.c. of inoculation represent an average of the two inoculations performed, whenever these occurred at different times. It will be observed that in all but one instance, an increase in inoculum was responsible for an increase in ammonia.

In order to determine what influence the additions of culture medium *per se* might have, a preliminary test was performed where increasing amounts of Cook's No. II fungi medium were added to 0.2, 1.0 and 5.0 c.c. of inoculum. It was found that 1 c.c. of the medium caused a stimulation in each case, but an increase beyond that amount did not result in increased ammonification. Thus 4 c.c. of medium added to 1 c.c. of spore-suspension gave no increase in ammonia over the addition of only 1 c.c. of medium to 1 c.c. of spore-suspension. Therefore, 5 c.c. of

TABLE V.  
COMPARISON OF AMMONIFICATION BY FUNGI, USING 0.2, 1.0 AND 5.0 C.C.  
OF SPORES FOR INOCULATION.

Fungus	Increase over check Av. Mg. N Dried Blood Series			Increase over check Av. Mg. N Cottonseed Meal Series		
	0.2 c.c.	1.0 c.c.	5.0 c.c.	0.2 c.c.	1.0 c.c.	5.0 c.c.
<i>Penicillium</i> sp. 10.....	3.45	12.62	23.91	6.40	17.71	28.71
<i>Rhizopus nigricans</i> .....	15.39	29.24	47.21	31.62	37.48	52.35
<i>Zygorrhynchus Vuilleminii</i> ...	5.78	6.89	8.54	23.15	26.27	50.29
<i>Rhizopus Oryzae</i> .....	16.14	18.94	23.01	31.11	28.53	33.16

Fungus	Percentage of maximum ammoni- fication with Dried Blood			Percentage of maximum ammoni- fication with Cottonseed Meal		
	0.2 c.c.	1.0 c.c.	5.0 c.c.	0.2 c.c.	1.0 c.c.	5.0 c.c.
<i>Penicillium</i> sp. 10.....	14.4	52.8	100	22.3	61.2	100
<i>Rhizopus nigricans</i> .....	32.6	61.9	100	60.3	71.6	100
<i>Zygorrhynchus Vuilleminii</i> ...	68.2	81.2	100	44.4	52.3	100
<i>Rhizopus Oryzae</i> .....	70.0	82.2	100	88.9	81.4	94.9
Average .....	46.3	69.5	100	54.0	66.6	98.7

spore-suspension would not carry a sufficient quantity of additional nutrients over that contained in 1 c.c. of spore-suspension, to affect the results seriously. From the lower half of Table V, where are summarized the data on inoculation with 0.2, 1.0 and 5.0 c.c. with regard to the percentage of maximum ammonification, it is evident that 0.2 c.c. caused a production of only 50 per cent of the maximum, while 1 c.c. was responsible for practically 70 per cent. Of course, 5 c.c. yielded the maximum. For all practical purposes, however, and in view of certain other factors to be considered in another connection, it is apparent that 1 c.c. of inoculum gives satisfactory results.

In order to facilitate the discussion of the results in Table V, the same data are presented in a different form in Table VI. The calculations are based upon the percentage increase in ammonia as a result of using 1 c.c. of inoculum compared with 0.2 c.c., and 5 c.c. as compared with 1 c.c. in the dried blood and cottonseed meal series, respectively. In other words, a 500 per cent increase in inoculum has been twice em-

ployed. In the dried blood series (with but one exception) the percentage increase in ammonia, when 1 c.c. is used as against 0.2 c.c., is greater than when 5 c.c. is compared with 1 c.c. The reverse apparently obtains in the cottonseed meal series (with one exception). This might be interpreted as signifying that dried blood is not as acceptable a source of food to these fungi as cottonseed meal, for with an increasing number of spores the competition seems to grow more keen. In point of fact, a casual glance at the relative ammonia accumulation in the tables of results with the different fungi appears to substantiate the observation that with cottonseed meal there is a somewhat greater ammonia accumulation than with dried blood. The last column of Table VI contains a general average of the increase in ammonia due to a 500 per cent increase in inoculum. In general, it might be stated that there was an increase of approximately 45 per cent (except for *Penicillium* which was considerably higher), or, in effect, the increase in inoculum by unit quantities, roughly speaking, increases the ammonia accumulation about one-tenth.

TABLE VI.  
PERCENTAGE INCREASE OF AMMONIFICATION BY FUNGI, USING 0.2, 1.0 AND 5 C.C. OF INOCULUM.

Fungus	Dried Blood Series		Cottonseed Meal Series		Av. In. due to 500% incr. in inoculum
	Incr. 1 c.c. over 2.0 c.c. %	Incr. 1 c.c. over 1.0 c.c. %	Incr. 5 c.c. over 0.2 c.c. %	Incr. 5 c.c. over 1.0 c.c. %	
<i>Penicillium</i> sp. 10.....	265	89	177	63	148
<i>Rhizopus nigricans</i> .....	62	40	14	35	38
<i>Zygorrhyncus Vuilleminii</i>	33	16	40	79	51
<i>Rhizopus Oryzae</i> .....	45	59	..	16	40

<sup>1</sup> Not averaged

The relation of the number of spores used in inoculation to ammonia accumulation has still another important aspect. It does not alone suffice to have determined the amount of inoculum to be used for maximum ammonia accumulation, but it is, moreover, prerequisite to further investigation with soil fungi to establish the optimum amount of inoculum which will bring out the differences which the individual organism manifests in its action upon various materials. Concretely, then, it is desirable to know whether 0.2, 1.0 or 5.0 c.c. for the sake of argument, will produce the greatest difference between the ammonification of dried blood and cottonseed meal respectively.

Some light is thrown upon this point in Table VII. It will be seen that with *Penicillium* 1.0 c.c. of inoculum gives the most noticeable difference between the ammonification of dried blood and cottonseed meal. However, 5 c.c. gives practically the same result. With *Rhizopus nigricans* 0.2 c.c. is superior to 1.0 c.c., which in turn is more striking in its effect than 5 c.c. With *Zygorrhyncus* it will be noted that 1 c.c. is su-

perior to 0.2 c.c., but inferior to 5 c.c. Possibly the poor ammonification of dried blood in this case exaggerates to some extent the large increase of 5 c.c. over 1 c.c. With *Rhizopus Oryzae*, 0.2 c.c. is superior to 1 c.c., which is almost equal to 5 c.c.

Thus considering all four organisms used, it is evident that in a majority of cases 1 c.c. is as efficient as 5 c.c. of inoculum. In effect, this implies that there is no advantage to be derived from increasing the number of spores in the inoculum beyond a certain point. Although the smallest quantity of spores, i. e. 0.2 c.c., was sufficient to bring out the difference between the ammonification of dried blood and cottonseed meal, there are several practical objections to its adoption. 1. Where such a small quantity is used there is an increase in experimental error based upon the technique of manipulation. 2. There is inaccuracy in the use of the pipette. 3. It is more difficult to obtain an equal distribution of spores in such a small sample.

TABLE VII.  
DIFFERENCES BETWEEN AMMONIFICATION OF DRIED BLOOD AND COTTONSEED MEAL BY FUNGI, USING 0.2, 1.0, AND 5.0 C.C. OF SPORES FOR INOCULATION.

Fungus	Difference between ammonification of Dried Blood and Cottonseed Meal Av. Mg. N.			No. of Spores per 1 c.c.
	0.2 c.c.	1.0 c.c.	5.0 c.c.	
<i>Penicillium</i> sp. 10 . . . . .	2.95	5.09	4.80	96,000
<i>Rhizopus nigricans</i> . . . . .	16.23	8.24	5.14	248,000
<i>Zygorrhynchus Vuilleminii</i> . . . . .	17.37	19.38	41.75	276,000
<i>Rhizopus Oryzae</i> . . . . .	14.97	9.59	10.15	88,000

In general, 1 c.c. is recommended as the most desirable quantity for inoculation for the following reasons. 1. One c.c. gave as striking differences as 5 c.c. 2. It is to be preferred to the latter because of economy of time. 3. Likewise it is preferable because economy of inoculating material. 4. Since less culture medium is added, there is consequently less possibility for the introduction of a disturbing factor.

Thus, in concluding this part of the investigation the salient points which have been established under the conditions of the experiment are as follows:

1. An increase in the number of spores inoculated into the soil is responsible for a proportional increase in ammonia accumulation.
2. Increasing the number of spores used in inoculation beyond a certain point does not further accentuate the difference between the ammonification of dried blood and cottonseed meal by these fungi.
3. One c.c. of spore suspension is the most desirable quantity to employ in soil fungi work.
4. Under the conditions of moisture and temperature employed, cottonseed meal is a more acceptable source of food for these fungi than dried blood.

## II.—STUDIES ON THE INCUBATION PERIOD OF ZYGORRHYNCUS VUILLEMINII AND RHIZOPUS NIGRICANS.

In ammonification studies with soil microorganisms an incubation period of 7 days has been quite generally adopted. While this point has been treated to a considerable degree in investigations concerning bacteria, such has not been the case with regard to soil fungi. Waksman and Cook (16) in studies with *Mucor plumbeus*, *Penicillium sp.* and *Monilia sitophila* found that the most practical duration for incubation was 12 days. In the present investigation the organisms under observation were *Zygorrhyncus Vuilleminii* and *Rhizopus nigricans*. The soil was the same as that used in the previous work, namely Norfolk sandy loam; and the organic nitrogenous materials, dried blood and cottonseed meal were added in amounts equivalent to 155 mg. of nitrogen. As before, the soil was made up to optimum moisture content and sterilized in 200 c.c. Erlenmeyer flasks at 15 pounds pressure for 15 minutes. Upon cooling, the soil in each flask was inoculated with 1 c.c. of spore-suspension of the desired organism (prepared according to the method already described). The flasks were then incubated at 18 to 21° C. throughout the duration of the experiment.

TABLE VIII.  
INCUBATION PERIOD OF ZYGORRHYNCUS VUILLEMINII.  
NORFOLK SANDY LOAM

Lab. No.	Days	Organic Matter	H <sub>2</sub> O c.c.	Mg. N.	Mg. N.	Av. Mg. N.	Increase over ch'k Mg. N.	Inc. over precd. day Mg. N.
91-92	Check	155 Mg. N. Dried Bld.	16.7	2.90	3.25	3.08	.	....
1-2	1	"	"	3.20	3.30	3.25	0.17	0.17
5-6	2	"	"	3.53	3.50	3.52	0.44	0.27
7-8	3	"	"	3.47	3.50	3.49	0.41	-0.03
11-12	4	"	"	4.66	4.60	4.63	1.55	1.14
14-15	5	"	"	10.14	9.27	9.71	6.63	6.08
17-18	6	"	"	19.92	20.05	19.99	16.91	10.28
19-21	7	"	"	22.10	22.25	22.18	19.10	2.19
23-24	8	"	"	32.55	31.10	31.83	28.75	9.65
25-27	9	"	"	30.60	30.90	30.75	27.67	-1.08
29-30	10	"	"	33.70	33.68	33.69	30.61	2.94
31-33	11	"	"	34.50	34.48	34.49	31.41	0.80
35-36	12	"	"	36.17	35.85	36.01	32.93	1.52
38-39	13	"	"	35.99	36.10	36.05	32.97	0.04
41-42	14	"	"	36.10	36.00	36.05	32.97	0
43-45	15	"	"	37.00	37.78	37.39	34.31	1.34

<sup>1</sup> Free ammonia liberated.

In Table VIII is presented the ammonia accumulated in the soil by *Zygorrhyncus Vuilleminii* acting on dried blood for 15 successive days. The column headed "increase over check in mg. N." which is graphically illustrated in figure 5 shows that there is practically no ammonification

until the 5th day, following which it takes place with increasing rapidity until the 8th day. After that time there is a slight increase (with one exception) until the 12th day, when a constant ensues. There is, then, good reason to believe that the maximum production of ammonia takes place

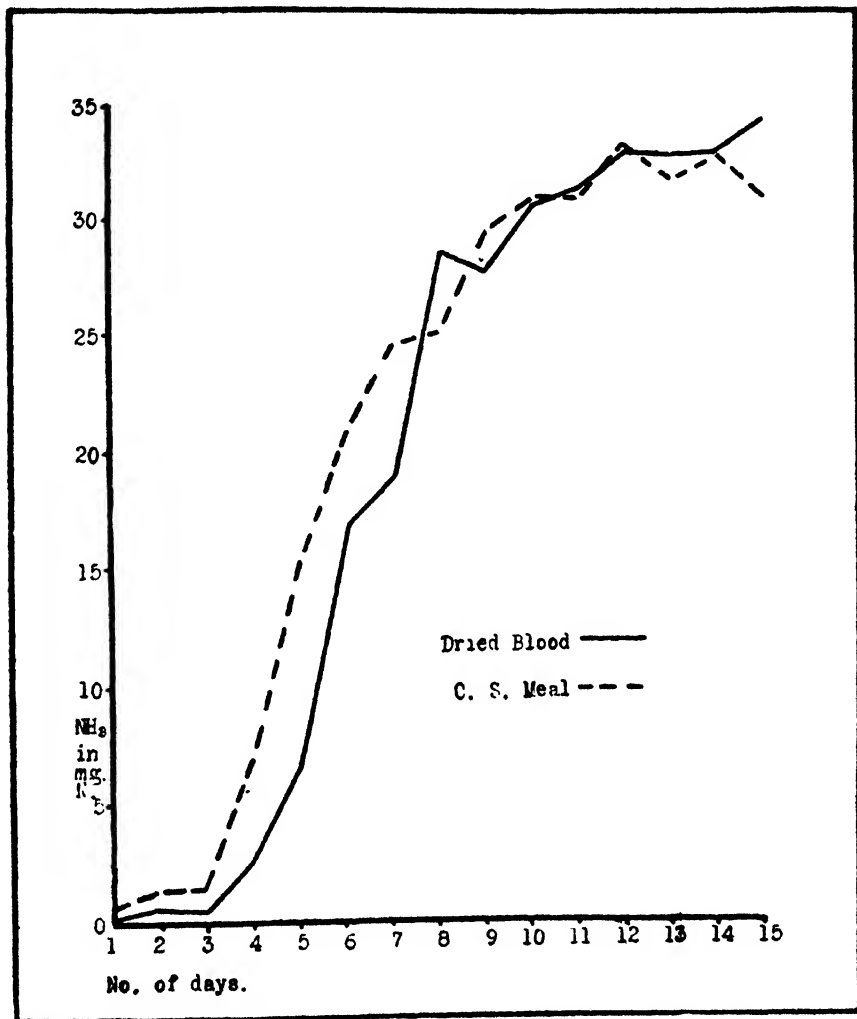


Fig. 5. Incubation period of *Zygorrhynchus Vuilleminii*. Increase over check of ammonia in mg. N.

between the 5th and 8th days. This might be interpreted as bearing out the theory advanced in discussing inoculation, namely that there is an increase in ammonia production with an increase in the number of spores, up to a certain point. (The number of spores may be seen to develop rapidly during the first week of growth.) On the other hand, an explanation of these results might depend, to even a greater extent, upon the metabolic processes involved.

It is interesting to note the daily increase in ammonia over the preceding day as shown in the last column of Table VIII and plotted in figure 6. It will be seen that there is a marked increase from the 4th to the 6th day, when the maximum gain is made. Thereafter there appears to be a singular fluctuation (up to the 12th day), where every other day marks a decided gain, the intervening days showing only slight increase. There has been an attempt made to correlate ammonia production with a biological stage of fungus, namely spore production and germination (15), but the above results do not seem to bear out such an hypothesis. The time elapsing between the germination of a spore of *Zygorrhyncus* and the appearance of sporangia on the mycelium developing from that spore is, roughly speaking, 3 days as determined by observation of the growth of the organisms in soil as well as in hanging drop preparations. Thus one would expect according to the theory just

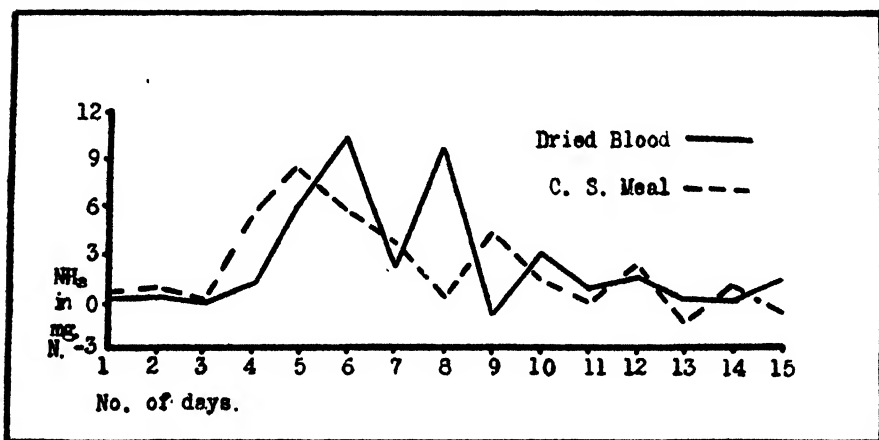


Fig. 6. Incubation period of *Zygorrhyncus Vuilleminii*. Daily increase in ammonia in mg. N.

set forth, that there would be a distinct increase in ammonia production every third day. But the data at hand points quite definitely to a sharp increase every *other* day. It appears to the writer that this phenomenon of fluctuation might more reasonably be interpreted in the light of the metabolic process of the fungus. In effect, ammonia accumulation, being a resultant of the concomitant factors of production and consumption, might fluctuate from day to day as a result of the predominance of first one and then the other of these two factors mentioned. Again, it will be noted that in one instance, namely, on the 9th day there was actually a decrease in ammonia as compared with the previous day. Undoubtedly, the production of spores is more or less continuous after the 4th day, therefore such a decrease seems to depend chiefly upon the metabolic processes rather than on any biological stage of a fungus.

In Table IX are recorded the results obtained in the ammonification of cottonseed meal by *Zygorrhyncus*. Upon directing attention to the column headed "increase over check in mg. N.," which is graphically illustrated in figure 5, it will be noted that there is very little ammonification up to the 4th day, after which there is a rapid increase to the 7th day. Subsequently there is a more variable increase up to the 12th day which marks the maximum. There is a decline after the 12th day. It will be observed that the maximum ammonia production occurs between the 4th and 9th days. In the last column of Table IX which represents the daily increase over the preceding day, which is graphically illustrated in figure 6, it will be seen that the greatest single increase occurred on the 5th day. Furthermore, the phenomenon of fluctuation, previously discussed, again becomes conspicuous after the 6th day. In other words, there is a sharp increase every other day followed by a less striking increase on the intervening days. On the 13th and 15th days, respectively, there was a decrease compared with the ammonia produced on the preceding day.

TABLE IX.  
INCUBATION PERIOD OF ZYGORRHYNCS VUILLEMINII.  
NORFOLK SANDY LOAM.

Lab. No.	Days	Organic Matter	H <sub>2</sub> O c.c.	Mg. N.	Mg. N.	Av Mg.N.	Increase over ch'k Mg. N.	Inc. over preced.day Mg. N.
		155 Mg. N. Cot'nseed Meal	20.5	3.85	3.75	3.80	.....	....
93-94	Check							
47-48	1	"	"	4.40	4.33	4.37	0.57	0.57
50-51	2	"	"	5.00	5.25	5.13	1.33	0.76
53-54	3	"	"	5.20	5.25	5.23	1.43	0.10
56-57	4	"	"	10.65	10.63	10.64	6.84	5.41
59-60	5	"	"	18.76	19.75	19.22	15.42	8.58
61-63	6	"	"	25.00	24.78	24.89	21.09	5.67
64-65	7	"	"	27.95	29.15	28.55	24.75	3.66
68-69	8	"	"	29.00	29.10	29.05	25.25	0.50
71-72	9	"	"	33.35	33.34	33.35	29.55	4.30
74-75	10	"	"	34.00	35.50	34.75	30.95	1.40
76-78	11	"	"	35.05	34.45	34.75	30.95	0
80-81	12	"	"	37.30	36.75	37.03	33.23	2.28
82-84	13	"	"	36.28	34.90	35.59	31.79	-1.44
85-86	14	"	"	37.30	35.90	36.60	32.80	1.01
88-89	15	"	"	36.55	32.68	34.98	31.18	-0.62

Comparing now the results obtained in the ammonification of dried blood (Table VIII), with that of cottonseed meal (Table IX) which may be seen quite readily in figure 5, it will be noted that the curves run practically parallel. Up to the 10th day (with one exception) the cottonseed meal is responsible for greater increases in ammonia than dried blood. This corroborates the conclusion arrived at in studies on inoculation, that for *Zygorrhyncus* cottonseed meal is a more acceptable source of food



than dried blood. Furthermore, it will be noted that cottonseed meal exhibits a fair production of ammonia on the 4th day, while this is delayed one day in the case of dried blood. From the 10th to the 14th day the curves are strikingly alike. The same is true of the results indicating the daily increase over the preceding day as shown in figure 6. It will be observed that the greatest single increase came on the 5th day in the case of cottonseed meal and on the 6th day with dried blood, which is the same as the relation which obtains between these two materials regarding the initial production of ammonia.

TABLE X.  
INCUBATION PERIOD OF RHIZOPUS NIGRICANS.  
NORFOLK SANDY LOAM.

Lab. No.	Days	Organic Matter	H <sub>2</sub> O c.c.	Mg. N.	Mg. N.	Av. Mg. N.	Increase over ch'k Mg. N.	Inc. over precd. day Mg. N.
142-143	Check	155 Mg. N. Dried Bld.	16.7	3.40	3 22	3 31	..	....
144-145	1	"	"	3.80	3.39	3.60	0.31	0.31
146-147	2	"	"	5.15	5.11	5.13	1.82	1.51
148-149	3	"	"	20.81	20 66	20.73	17.42	15.60
150-151	4	"	"	27.74	27.15	27.43	24 12	6.70
152-153	5	"	"	31 06	31.00	31.03	28.72	4.60
154-155	6	"	"	29.74	34.31	32 02	29.71	0.99
156-157	7	"	"	42.50	45.31	43.95	40.64	10.93
158-159	8	"	"	35.31	35.42	35.36	32.05	—8.59
160-161	9	"	"	37.05	36.30	36.67	33.36	1.31
162-163	10	"	"	39.43	39.28	39.35	136.04	12.68
164-165	11	"	"	57.77	53.42	55.59	152 28	116.24

<sup>1</sup> Liberation of free ammonia.

Considering the influence of incubation period on the ammonification of dried blood by *Rhizopus nigricans* as shown in Table X and graphically illustrated in figure 7, it will be noted that there is practically no production of ammonia until the 3rd day, after which there is an increase up to the 7th day when a maximum is attained. After the 7th day there is a decline until free ammonia is liberated, as evidenced by odor and the litmus paper test. It was deemed unnecessary to continue the experiment for a longer period of time. Compared with *Zygorrhyncus*, this organism produces ammonia much more rapidly and attains its maximum in a shorter period of time. In hanging drop preparations the spores germinate within 24 hours. In considering the daily increase over the preceding day it will be observed that the maximum occurs on the 3rd day, or 3 days earlier than with *Zygorrhyncus*. From the 5th day on, the phenomenon of fluctuation previously noted, is apparent. As shown in figure 8, this is rather striking.

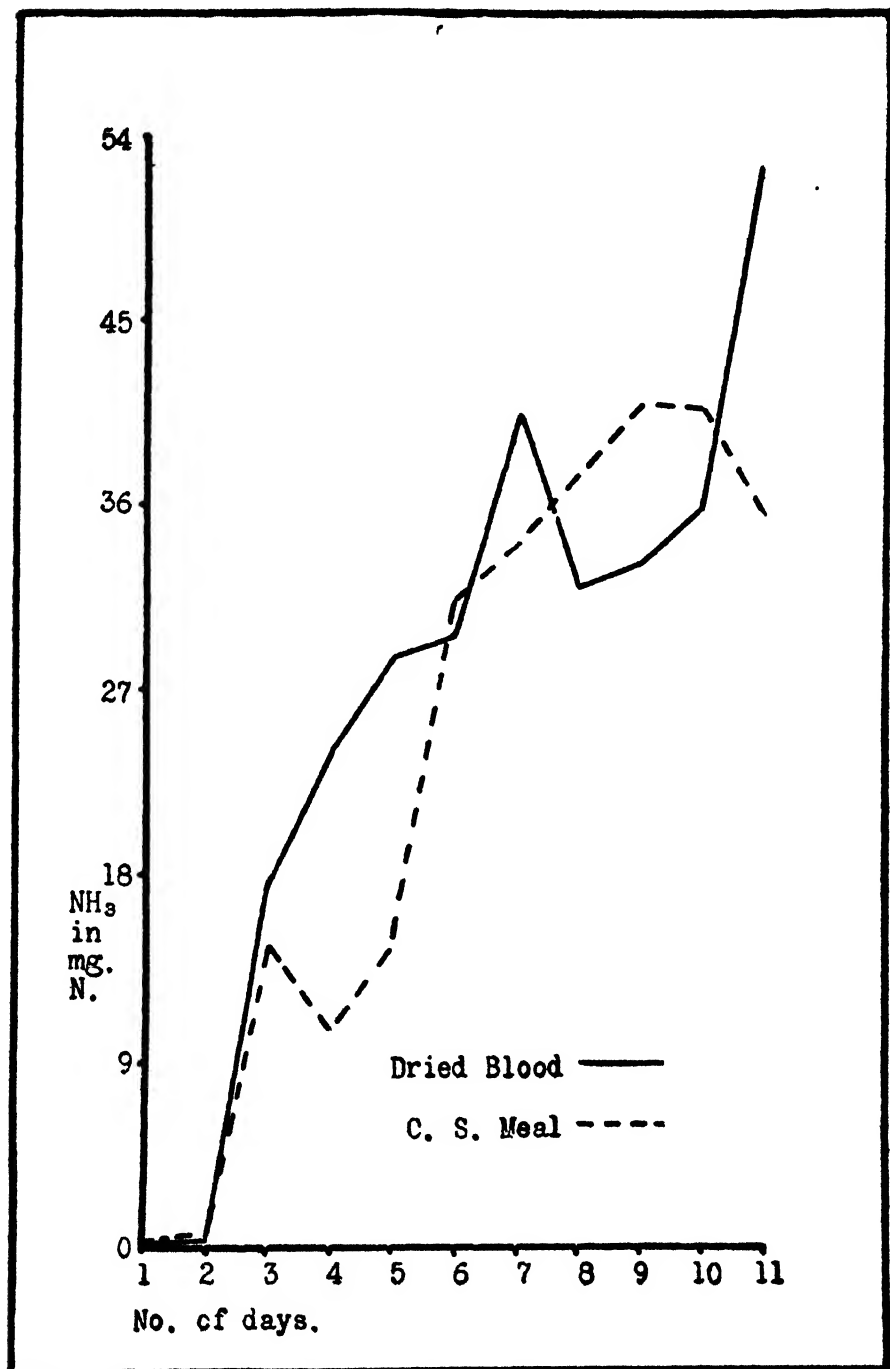


Fig. 7. Incubation of *Rhizopus nigricans*. Increase over check of ammonia in mg. N.

In Table XI and figure 7, may be seen the increase in ammonia accumulation where cottonseed meal is used as a source of organic nitrogenous matter. Again, as in the case of dried blood, the production of ammonia does not become vigorous until the 3rd day, and increases up to the 9th day when a maximum is attained. Thereafter a decline sets in, which occurs two days later than in the case of dried blood.

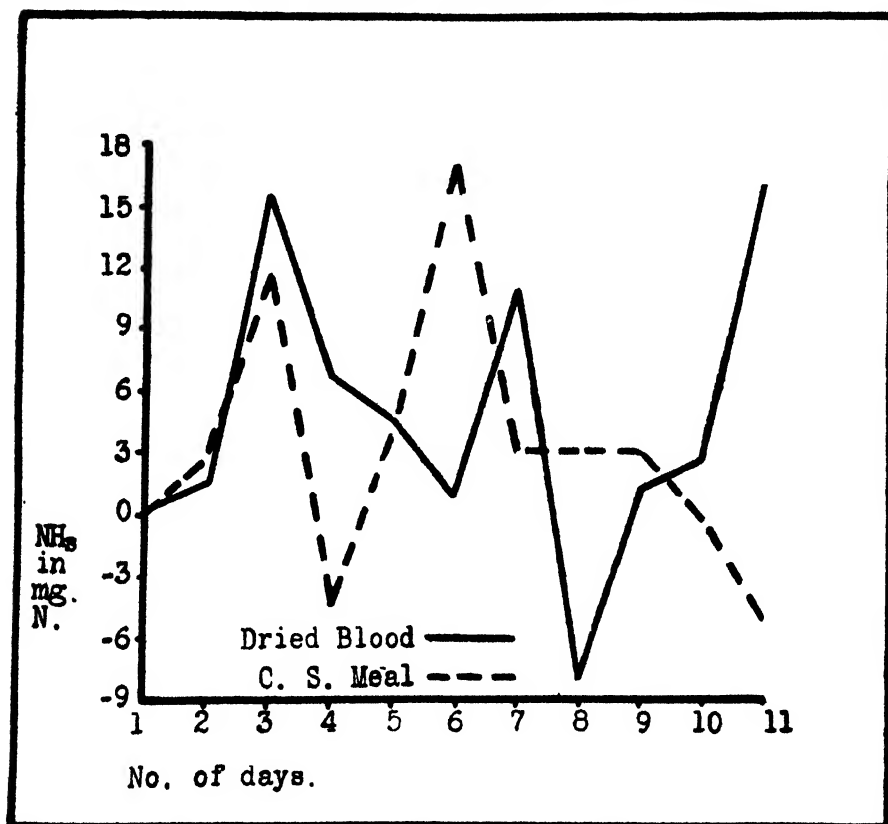


Fig. 8. Incubation period of *Rhizopus nigricans*. Daily increase in ammonia in mg. N.

With regard to the daily increase over the preceding day as shown in the last column of Table XI and in figure 8, a large increase occurs on the 3rd day, while a still larger appears on the 6th day. The fluctuation phenomenon is not as distinct as in previous cases, but the general tendency seems to point in the same direction.

To recapitulate, the maximum ammonification of dried blood and cottonseed meal by *Zygorrhynchus* occurs on the 12th day, while with *Rhizopus nigricans* the maximum ammonia accumulation with dried blood occurs on the 7th day and with cottonseed meal on the 9th day.

As in the case of inoculation, so likewise with incubation, it is essential to determine what period is best adapted to showing the difference between the ammonification of dried blood and of cottonseed meal.

TABLE XI.  
INCUBATION PERIOD OF RHIZOPUS NIGRICANS.  
NORFOLK SANDY LOAM.

Lab. No.	Days	Organic Matter	H <sub>2</sub> O c.c.	Mg. N.	Mg. %	Av. Mg. N.	Increase over ch'k Mg. N.	Inc. over precd. day Mg. N.
		155 Mg. N. Cot'nseed Meal	20.5	3.69	3.69	3.69	...	....
165-166	Check			3.65	3.65	3.65	0	0
167-168	1	"	"	6.49	6.64	6.56	2.87	2.87
169-170	2	"	"	18.02	18.71	18.36	14.76	11.89
171-172	3	"	"	15.97	12.39	14.18	10.49	—4.27
173-174	4	"	"	21.69	14.46	18.07	14.38	3.89
175-176	5	"	"	35.27	35.07	35.17	31.48	17.10
177-178	6	"	"	38.52	38.08	38.30	34.61	3.13
179-180	7	"	"	41.46	41.40	41.43	37.70	3.09
181-182	8	"	"	44.91	44.18	44.59	40.90	3.20
183-184	9	"	"	44.45	44.49	44.47	40.78	—0.12
185-186	10	"	"	39.99	38.83	39.41	35.72	—5.06
187-188	11	"	"					

Table XII has been prepared with this point in view, the figures recorded represent the remainder after subtracting the amount of ammonia accumulated with one organic matter from that accumulated by the other. It will be noted that with both fungi used, the 7-day incuba-

TABLE XII.  
DIFFERENCES IN AMMONIFICATION BY SOIL FUNGI OF DRIED BLOOD COMPARED WITH COTTONSEED MEAL, AT 3, 7, 10 AND 15 DAYS' INCUBATION.

Organism	Differences between Ammonia accumulated in presence of Dried Blood and Cottonseed Meal (Increase over check av. mg. N.)		
	3 days	7 days	10 days
<i>Zygorrhynchus Vuilleminii</i> .....	1.02	5.65	0.34
<i>Rhizopus nigricans</i> .....	2.66	6.03	4.74

	Per cent of Maximum Ammonification					
	D. Bld.	C. S. M.	D. Bld.	C. S. M.	D. Bld.	C. S. M.
<i>Zygorrhynchus Vuilleminii</i> .....	1	4.2	57.9	75.1	93.9	84.0
<i>Rhizopus nigricans</i> .....	42.8	36.2	100.0	84.6	90.0	99.5

tion period showed the most striking differences. Furthermore it will be seen from the lower half of table XII that with regard to the percentage of maximum ammonification, the 3-day period cannot be considered adequate, while the 7-day period is not markedly inferior to the 10-day period. Since the 7-day incubation period involves less risk of loss of free ammonia from the soil as well as evaporation of moisture, and repre-

sents an economy of time, it is to be recommended as the most desirable incubation period for the soil fungi considered. From observation, it is also suggested that this would hold true for most other groups of soil fungi, with the exception of the important *Penicillia* and possibly some others.

#### SUMMARY.

Under the conditions of the experiment the following points may be noted in studies with *Penicillium* sp. 10, *Rhizopus nigricans*, *Zygorrhyncus Vuilleminii*, and *Rhizopus Oryzae*, in Norfolk sandy loam.

1. An increase in the number of fungi spores inoculated into the soil is responsible for a proportional increase in ammonia accumulation.

2. One c.c. of spore-suspension, all factors considered, is the most desirable quantity for inoculation in experiment with pure cultures of soil fungi.

3. Increasing the number of spores used in inoculation beyond a certain point does not further accentuate the difference between the ammonification of dried blood and cottonseed meal by these fungi.

4. Under the conditions of moisture and temperature employed, it appears that cottonseed meal is a more acceptable source of food than dried blood for the organisms studied.

5. With *Zygorrhyncus Vuilleminii* the maximum ammonia accumulation occurs on the 12th day with both kinds of organic matter. With dried blood *Rhizopus nigricans* yields the maximum on the 7th day and with cottonseed meal on the 9th day.

6. All factors considered, a 7-day incubation period may be recommended as most desirable for the study of soil fungi, other than those belonging to the *Penicillium* group.

7. There is a striking increase in ammonia production taking place every other day (after the first five days).

8. There is good reason to believe that the production of ammonia is dependent on the metabolic processes of the fungus rather than the biological stage of spore production and germination.

In conclusion it is a privilege to thank Dr. J. G. Lipman for his helpful suggestions ever at the writer's disposal.

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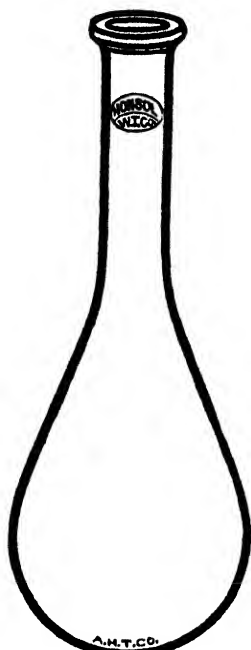
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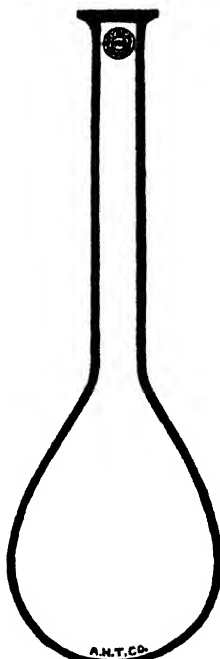
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RUTGERS COLLEGE

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## THE LOESS SOILS OF THE NEBRASKA PORTION OF THE TRANSITION REGION :

### IV. MECHANICAL COMPOSITION AND INORGANIC CONSTITUENTS'

By

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*Research Assistant in Soils, Agricultural Experiment Station,*  
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#### INTRODUCTION

In Nebraska the loess extends westward for about 300 miles from the eastern boundary on the Missouri River. Throughout this distance the temperature conditions are quite uniform, but there is a gradual decrease in the humidity of the climate, the normal annual precipitation, which exceeds 30 inches at the eastern boundary, steadily falling until it is less than 20 in the extreme western portion, while the rate of evaporation increases considerably. The climate of this region has been considered in detail in a previous paper (2, p. 206).

The soil samples, upon which this article is based, were collected from 30 virgin prairie fields, 5 near each of six stations of the United States Weather Bureau shown in figure 1—Wauneta, McCook, Holdrege, Hastings, Lincoln, and Weeping Water. In each field, at intervals of 30 feet, 10 borings were made to a depth of 6 feet and composite samples prepared of each foot-section, thus giving 6 samples from each field, the so-called "field-samples." From these "area-samples" were prepared for analysis by mixing equal weights of the corresponding five field samples. Thus each of the samples analyzed is a composite from 50 individual borings. The details of the method of sampling are given in the article referred to above.

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<sup>1</sup> Received for publication February 10, 1916.

<sup>2</sup> The work reported in this paper was carried out at the Nebraska Agricultural Experiment Station, where the authors were Chemist and Assistant in Chemistry, respectively.



## MECHANICAL ANALYSIS.

Mechanical analyses of the area samples were made by the methods of the Bureau of Soils (4, 8). Deflocculation was effected by 7 hours' shaking of 5 gm. of soil with 75 c.c. of water to which there had been added 2 c.c. of ammonia solution. The clay was determined by difference. The organic matter was allowed to remain in the separates, being distributed among the different fractions. Only in the case of the coarse sand was it determined by ignition. This fraction from the surface foot samples showed root fragments, but in no sample did the organic matter in this exceed 0.2 per cent of the weight of the soil. As all the soils consisted chiefly of silt and very fine sand, the two fractions most difficult to separate satisfactorily, especially as so much of the very fine sand was but little coarser than silt, we were very careful to make the separations as

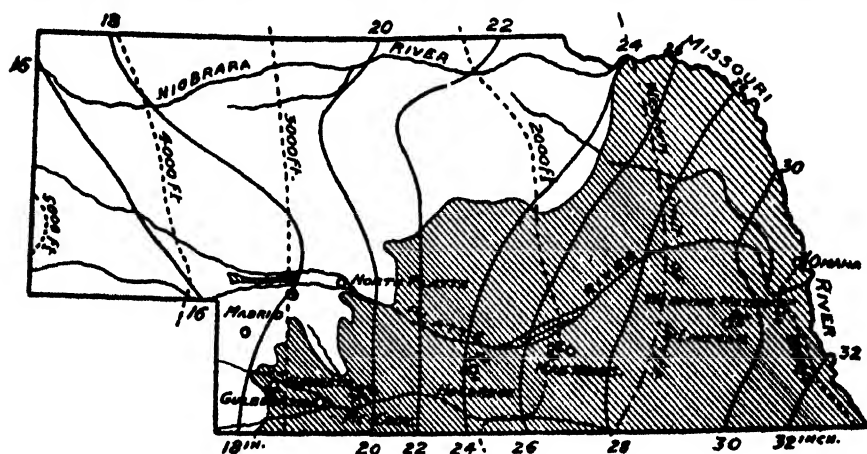


Fig. 1—Map of Nebraska showing distribution of the loess (shaded), annual precipitation and location of the fields sampled.

nearly alike as possible, all being carried out side by side. As a final precaution we compared under the microscope the corresponding fractions from the different samples, both before and after being dried.

The mechanical composition is shown in Table I and figure 2. All the samples consist chiefly of silt and very fine sand, the sum of these in no case being less than 77 or more than 95 per cent, it being highest in the most westerly three areas where it varies between 83.5 and 94.7 per cent. In the most easterly two it lies between the somewhat lower limits of 77.3 and 82.6 per cent, the decrease being compensated for by a corresponding increase in the amount of clay, which reaches a maximum of nearly 20 per cent in the eastern areas. The four fractions coarser than very fine sand together form only from 0.4 to 4.9 per cent, the fine sand constitu-

TABLE I  
MECHANICAL ANALYSIS OF FOOT-SAMPLES FROM THE DIFFERENT AREAS

## WAUNETA

Depth Foot	Fine Gravel 2.0-1.0 mm. %	Coarse Sand 1.0-0.5 mm. %	Medium Sand 0.5-0.25 mm. %	Fine Sand 0.25-0.10 mm. %	Very Fine Sand 0.10-0.05 mm. %	Silt 0.5-0.005 mm. %	Clay 0.005-0.000 mm. %
1	0.0	0.1	1.0	3.7	48.7	41.2	5.4
2	0.0	0.1	0.5	1.8	47.8	43.3	6.6
3	0.0	0.1	0.3	1.6	46.8	43.8	7.5
4	0.0	0.1	0.0	1.6	47.6	41.3	9.5
5	0.0	0.0	0.1	1.4	50.0	43.6	4.9
6	0.0	0.1	0.1	1.1	54.9	39.8	4.2
Average	0.0	0.1	0.3	1.9	49.3	42.2	6.3

## McCOOK

1	0.0	0.3	0.8	2.6	39.0	48.6	8.7
2	0.0	0.0	1.1	1.5	37.8	50.1	9.5
3	0.0	0.1	0.1	1.1	36.4	53.9	8.4
4	0.0	0.1	0.1	1.2	38.9	52.4	7.4
5	0.0	0.1	0.1	1.6	39.3	52.6	6.3
6	0.0	0.1	0.1	1.0	40.4	51.8	6.6
Average	0.0	0.1	0.4	1.5	38.6	51.6	7.8

## HOLDREGE

1	0.0	0.3	0.4	2.1	25.9	64.6	6.7
2	0.0	0.1	0.3	0.9	24.6	62.9	11.2
3	0.0	0.1	0.1	0.6	26.2	62.5	10.5
4	0.0	0.2	0.1	0.6	27.8	64.8	6.4
5	0.0	0.2	0.4	1.9	31.7	60.0	5.8
6	0.0	0.2	0.4	1.8	31.1	60.7	5.8
Average	0.0	0.2	0.3	1.3	27.9	62.6	7.7

## HASTINGS

1	0.0	0.3	0.7	2.9	23.9	64.6	7.6
2	0.0	0.0	0.5	2.3	20.3	64.5	12.5
3	0.0	0.0	0.6	1.7	22.2	61.9	13.6
4	0.0	0.2	0.4	1.5	21.5	62.4	14.0
5	0.0	0.2	0.5	1.7	20.9	66.7	10.0
6	0.0	0.3	0.4	1.5	20.7	67.2	9.9
Average	0.0	0.2	0.5	1.9	21.6	64.5	11.3

## LINCOLN

1	0.0	0.3	0.6	2.9	13.5	68.0	14.8
2	0.0	0.3	0.7	2.7	9.8	67.6	18.9
3	0.0	0.4	0.7	2.3	9.3	68.0	19.3
4	0.0	0.4	0.7	2.1	9.6	68.1	18.9
5	0.0	0.8	0.8	2.1	9.9	69.4	17.0
6	0.2	0.5	0.9	2.3	9.5	70.2	16.5
Average	0.1	0.4	0.7	2.4	10.3	68.5	17.6

## WEEPING WATER

1	0.0	0.5	0.7	3.0	9.7	72.2	13.9
2	0.0	0.1	0.5	2.2	8.2	69.5	19.6
3	0.0	0.1	0.1	0.8	13.8	66.7	18.6
4	0.0	0.1	0.1	0.4	14.9	67.0	17.6
5	0.0	0.1	0.1	0.3	14.7	67.9	17.0
6	0.0	0.1	0.1	0.3	15.0	67.5	17.1
Average	0.0	0.2	0.3	1.2	12.7	68.5	17.3
Av. of all Samples	0.0	0.2	0.4	1.7	26.7	59.7	11.3

ting the most of this. In only the sixth foot of the Lincoln area were any particles coarser than 1.0 mm. found. These came from Field III in which the loess was thinnest, and appear to have been derived from a boring that had barely penetrated the underlying Kansan till.

It will be seen that the amounts of the chief constituents, silt and very fine sand, show no distinct dependence upon the depth. In all the areas the proportion of clay is lower in the first than in the second foot, and shows a maximum in the second, third or fourth foot. The fine sand, low at all depths, is, in general, present in largest amount in the first foot.

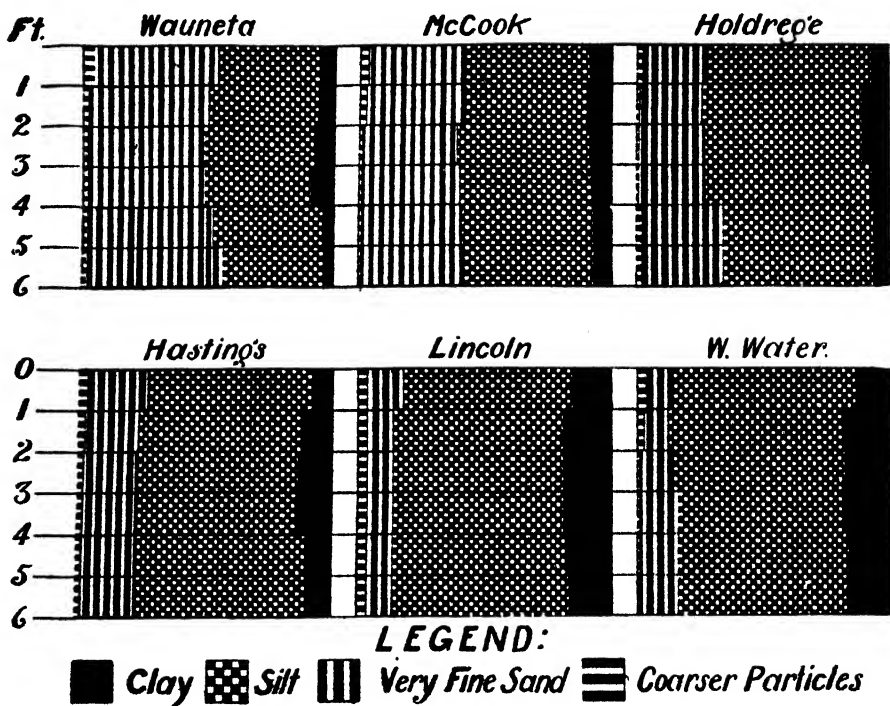


Fig. 2—Diagram showing the mechanical composition of the composite samples from the different areas.

As we pass from west to east the texture of the loess becomes finer. The proportions of the fractions coarser than 0.1 mm. are quite similar in all the areas, but those of both clay and silt are highest, and those of very fine sand lowest, in the eastern areas. However, when we compare the samples from two adjacent areas we do not find a regular increase or decrease in the amount of the different separates on passing from west to east. The Wauneta and McCook samples are quite similar, as are also those from Lincoln and Weeping Water, while the soils from the two intervening areas are intermediate in texture. The very fine sand shows

a steady decrease from Wauneta to Lincoln and then rises somewhat in the Weeping Water area. The silt rises from Wauneta to Holdrege and Hastings and again at Lincoln and Weeping Water. The clay is similar in amount at Wauneta and McCook, being low at both, is higher in the second and third foot at Holdrege, still higher at Hastings and much higher at both Lincoln and Weeping Water.

The Bureau of Soils of the United States Department of Agriculture<sup>1</sup> has reported the mechanical analyses of 20 samples of loess soils from Nebraska. None of these was taken from any point farther west than Holdrege. Half of them were surface soils taken to a depth of 12 to 16 inches, while the others were the corresponding subsoils, taken to represent the section between the surface sample and a depth of 36 inches. The analyses of these, only a few of which were composites, are on the whole quite concordant with those reported in Table I, if we omit from the comparison the data from the Wauneta area.

At the time our samples were collected only the fields near Lincoln had been included in a soil survey. Since then those of the westerly four areas have been covered by a reconnoissance survey of Western Nebraska (7) and those near Weeping Water by a detailed survey.<sup>2</sup> All the fields at Wauneta, McCook, Holdrege and Hastings from which the samples were taken are indicated on the map of the reconnoissance survey as Colby silt loam—"An ashy-gray to brownish-gray silt loam with a small content of fine sand and clay, ranging in depth from 6 to 24 inches. . . . The type is of wind-laid origin and is derived from the weathering of loess" (15, p. 427).

The fields at Lincoln were selected from the areas indicated on the soil map of Lancaster County as Marshall silt loam. Of the five fields at Weeping Water the Bureau of Soils, on the map in its recent report, indicates two, IV and V, as Marshall silt loam, but the other three as Shelby silt loam. The former is described as "a dark brown to black silt loam, 15 inches deep, resting usually upon a light-colored, sometimes mottled, silt loam or silty clay. . . . The soil is derived from loessial deposits" (15, p. 158). The latter is "a dark-brown or dark grayish brown, heavy silt loam 8 to 15 inches deep, underlain by a light-brown or yellowish brown, compact silty clay. . . . The nearness of the Kansan drift to the surface varies with the topographic position of this soil. . . . The divides are covered with a thick mantle of silt. The Shelby and Marshall silt loams are very similar in this county and differ mainly in point of origin. The Shelby silt loam is derived from the weathered phase of the

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<sup>1</sup> Soil Survey of the Stanton Area, 1904, Grand Island Area, 1904, Kearney Area, 1904, Sarpy Area, 1906, Lancaster Area, 1908.

<sup>2</sup> Soil Survey of Cass County, Nebraska, Bureau of Soils, U. S. D. A., 1914.

Kansan drift, whereas the Marshall silt loam is derived from loess. The latter carries no pebbles or boulders; the former a little of such coarse materials. . . . These two types grade imperceptibly into each other, and the boundary between them is necessarily largely arbitrary."<sup>1</sup> In the latter report it is stated (p. 28) that "the Shelby silt loam also includes small areas of Marshall silt loam, which, owing to their close similarity to the former cannot be satisfactorily separated."

In order to decide whether we were in error in selecting the fields as all typical of the loess we have determined the amount of coarse and medium sands in each foot-sample from the three of our fields indicated on the Bureau of Soils Map as Shelby silt loam, and find the amount no higher than in the other two (Table II). This together with the fact that no gravel was found in any may be considered as conclusive evidence that no glacial drift was included in any of the samples, and that the soil of these fields has been derived from the loess and not from Kansan till.

TABLE II  
COARSE AND MEDIUM SAND IN FIELDS AT WEEPING WATER

Depth	Field I	Field II	Field III	Fields IV & V
Foot	%	%	%	%
1	.3	.7	5	1.1
2	.4	.5	.5	.8
3	.4	.6	.1	1
4	.3	.3	2	1
5	.1	.1	1	1
6	.1	.1	2	.1

#### RELATION OF HYGROSCOPICITY TO MECHANICAL COMPOSITION

The hygroscopic coefficients of the area samples have been previously reported (2,p.216). Using the formula proposed by Briggs and Schantz (6, p. 73)—Hygroscopic coefficient = 0.007 sands + 0.082 silt + 0.39 clay—we have calculated the values from the data in Table I. These as well as the coefficients obtained by direct determination are reported in Table III. In the case of the Weeping Water and Lincoln samples the values obtained by the two methods agree satisfactorily, but a gradually increasing divergence is to be observed as we proceed westward from Lincoln. This corresponds to the increasing proportion of very fine sand and we find that by replacing the value 0.007, which Briggs and Shantz assign to all the sands, by 0.07 for very fine sand and by 0.005 for the coarser fractions we obtain a formula which applies equally well to the samples from all the areas. Hygroscopic coefficient = 0.005 coarser fractions + 0.07 very fine sand + 0.082 silt + 0.39 clay. In this modified formula the very fine sand is given a value almost as high as the silt. The material in the loess which falls into the former fraction consists chiefly of particles

<sup>1</sup> Soil Survey of Cass County, Nebraska, p. 27 to 29.

with a diameter but little in excess of 0.05 mm. and which, accordingly, differ but slightly from the silt particles. The effect both of the fine sand and of the coarser fractions might be ignored in such a calculation without the results being appreciably affected. We have found that dune sand, consisting of about 97 per cent of these fractions, has a hygroscopic coefficient of 0.4 or 0.5. For this reason in the proposed formula we assign the value 0.005 to the fractions coarser than 0.10 mm.

TABLE III

COMPARISON OF THE DETERMINED HYGROSCOPIC COEFFICIENTS WITH THE VALUES FOUND BY COMPUTATION FROM THE MECHANICAL ANALYSES:  
A. BY FORMULA OF BRIGGS AND SHANTZ; B. BY A MODIFIED FORMULA

Depth Foot	WAUNETA			McCOOK			HOLDREGE		
	Det'd	Computed		Det'd	Computed		Det'd	Computed	
		A %	B %		A %	B %		A %	B %
1	9.1	5.8	8.9	10.0	7.5	10.1	10.1	8.1	9.7
2	9.6	6.5	9.5	10.9	8.1	10.5	11.2	9.7	11.2
3	9.7	6.8	9.8	10.7	8.0	10.3	11.3	9.4	11.1
4	9.9	7.4	10.4	9.7	7.4	9.9	10.2	8.0	9.8
5	9.0	5.8	9.0	9.1	7.1	9.5	9.6	7.4	9.4
6	8.3	5.2	8.7	9.1	7.0	9.6	9.4	7.4	9.4
Av.	9.3	6.3	9.4	9.9	7.5	10.0	10.3	8.3	10.1

HASTINGS				LINCOLN			WEEPING WATER		
1	9.6	8.5	9.9	12.0	11.5	12.3	12.1	11.4	12.0
2	11.6	10.3	11.6	14.4	13.0	13.6	13.7	13.4	13.9
3	12.4	10.6	11.9	13.6	13.1	13.7	13.9	12.8	13.7
4	11.1	10.7	12.1	13.0	13.0	13.6	13.0	12.5	13.4
5	10.7	9.5	10.8	12.8	12.4	13.0	12.6	12.3	13.2
6	10.7	9.4	10.8	12.7	12.2	12.9	12.5	12.3	13.2
Av.	11.0	9.8	11.2	13.1	12.5	13.2	13.0	12.4	13.2

As an illustration of the significance in field studies of soil moisture of the difference between the actual hygroscopic coefficients and those computed by the Briggs and Shantz formula, assuming an average difference of 3.0, as found with the Wauneta samples, we may cite the instance of a neglected old orchard near McCook which was, during a very dry period in 1912, succumbing to drought. In the first six feet there was an average of only 1.1 per cent of free water (Table IV), while the calculated values of the hygroscopic coefficients would have indicated 4.1 per cent. However, under favorable moisture conditions, the same soil would carry from 8 to 15 per cent free water, and then the difference between the actual amount and that calculated by the Briggs-Shantz formula would have little significance.

## METHODS OF CHEMICAL ANALYSIS

All of the area-samples were subjected to a complete or rock analysis and also to extraction with strong hydrochloric acid. In the case of the "field samples" determinations of only the carbon dioxide were made.

In the complete analyses we followed the methods in use in the laboratory of the United States Geological Survey (13), except in the case of manganese. In determining this element by the method used in that laboratory we obtained discordant results. A new method was then developed (9) which we have used with all the samples.

TABLE IV

MOISTURE CONDITIONS IN AN OLD ORCHARD NEAR McCOOK, NEBRASKA, ON JUNE 27, 1912. AT THE TIME OF SAMPLING THE TREES WERE SUCCUMBING TO DROUGHT

Depth Foot	Total Water %	Hygroscopic Coefficient		Free Water	
		A Determined %	B Calculated %	By A %	By B %
1	10.2	8.4	5.4	1.8	4.8
2	13.0	10.6	7.6	2.4	5.4
3	10.7	9.8	6.8	0.9	3.9
4	9.5	8.9	5.9	0.6	3.6
5	9.3	8.9	5.9	0.4	3.4
6	8.9	8.3	5.3	0.6	3.6
Average	10.3	9.1	6.1	1.1	4.1

The determination of the acid-soluble constituents—the so-called zeolithic portion—was made by what is essentially Hilgard's method (11, p. 16). The soil was digested for 120 hours on the steam bath with hydrochloric acid of 1.115 specific gravity, after which the acid extract was analyzed by the methods recommended by the Association of Official Agricultural Chemists (3, p. 15) except that manganese was determined by the Gortner-Rost method mentioned above.

All analytical data reported are the averages of concordant duplicate determinations, except in the case of the silica, alumina, magnesia, lime and titanium oxide of the rock analyses. In the case of such complete analyses a partial control is provided by the magnitude of the departure of the sum of the constituents from 100 per cent.

The distribution of the different inorganic constituents is reported in Tables V to XVIII. The first part of each table shows the total amount of the constituent, the second, where given, the amount dissolved by 5 days' digestion with strong hydrochloric acid, and the third the portion remaining undissolved. The data in the last are not the result of direct determinations, they being obtained by subtracting the values in the second part from those in the first. Baryta was not found in the acid ex-

tracts and hence only the total is reported. The sum of the constituents remaining undissolved should approximate, but not necessarily be identical with, the amount of insoluble matter found by digestion, as the difference between the two represents the summation of departures for the eleven soluble constituents.

## LIME

The lime (Table V) shows greater variations than any other constituent, except the carbon dioxide. The total amount rises from east to west, being three times as high in the lower levels of the most westerly two areas as in those of the most easterly two. In the former it is much lower in the first and second foot than in the underlying levels, but in the

TABLE V  
LIME IN THE FOOT-SECTIONS

## TOTAL

Depth Foot	Wauneta %	McCook %	Holdrege %	Hastings %	Lincoln %	W. Water %	Average %
1	1.67	1.40	1.18	1.13	.72	.89	1.16
2	1.69	1.80	1.33	1.16	1.00	.95	1.32
3	2.72	3.59	1.60	1.45	1.21	1.37	1.99
4	3.93	3.91	2.15	1.75	1.21	1.08	2.34
5	3.91	3.54	2.30	1.80	1.39	1.08	2.34
6	3.94	3.44	2.19	1.72	1.31	1.18	2.30
Average	2.98	2.95	1.79	1.50	1.14	1.09	1.91

## ACID SOLUBLE

1	1.10	.88	.74	.68	.61	.68	.78
2	1.15	1.38	.86	.74	.64	.71	.91
3	1.81	3.03	.97	.97	.75	.76	1.38
4	3.06	3.33	1.69	1.22	.90	.78	1.83
5	3.04	3.04	1.84	1.33	1.10	.78	1.85
6	3.43	2.84	1.84	1.36	.96	.99	1.90
Average	2.26	2.42	1.32	1.05	.83	.78	1.44

## ACID-INSOLUBLE

1	.57	.52	.44	.45	.11	.21	.38
2	.54	.42	.47	.42	.36	.24	.41
3	.91	.56	.63	.48	.46	.61	.61
4	.87	.58	.46	.53	.31	.30	.51
5	.87	.50	.46	.47	.29	.30	.48
6	.51	.60	.35	.36	.35	.19	.39
Average	.72	.53	.47	.45	.31	.31	.46

latter it shows no direct dependence upon the depth. The Holdrege and Hastings soils in this, as in almost every other respect, show a behavior intermediate between that of the two areas to the west and that of the two to the east. This marked variation is due chiefly to the acid-soluble portion. The insoluble part shows no distinct relation to the depth, but rises somewhat from east to west.



## CARBON DIOXIDE

The variation in the carbon dioxide (Table VI) agrees with that of the acid-soluble lime, varying from an amount too small to be determined by ordinary methods to 2.34 per cent. Carbonates are practically absent from the first foot of all the fields, and from the second foot also, except in the case of three fields at McCook, the amounts found being within the range of experimental error.

TABLE VI  
CARBON DIOXIDE IN THE FOOT-SECTIONS.

Depth Foot	Wauneta %	McCook %	Holdrege %	Hastings %	Lincoln %	W. Water %	Average %
1	.09	.02	.01	.01	.01	.01	.02
2	.07	.40	.03	.02	.02	.01	.09
3	.59	2.02	.13	.10	.02	.05	.48
4	1.67	2.34	.75	.36	.06	.00	.87
5	1.78	2.11	1.00	.38	.12	.02	.90
6	1.68	2.08	1.05	.41	.08	.01	.88
Average	.98	1.49	.49	.21	.05	.02	.54

The subsoils of the Weeping Water area show no carbonates. At Lincoln an appreciable amount is found only in the lower levels, where it occurs in the form of small concretions of calcium carbonate distributed through the fourth, the fifth and the sixth foot. In the lower levels at Hastings it occurs in considerable quantities, at Holdrege in large amounts and in the McCook and Wauneta fields it is still more abundant. In the western areas the carbonates are distributed throughout the subsoil mass instead of being segregated in the form of concretions. As it is important to know whether these differences in the amount of carbonates in the lower levels are common to all the fields of the areas concerned, we determined the carbon dioxide in the case of all the fields at Wauneta, McCook and Holdrege, except where the analysis of the composite had shown a negligible amount (Table VII). All the fields of these three areas show a high content of carbonates (0.33 to 3.05 per cent  $\text{CO}_2$ ) in the fourth, the fifth and the sixth foot. The amount in the third foot is much more variable, while that in the first foot of all of the fields and in the second of all except three at McCook is negligible. The differences between fields in the same area is too great to permit of the carbon dioxide content serving as a definite area characteristic although it distinguishes the subsoils of the eastern portion of the loess from those of the central and western portions.

## MAGNESIA

The magnesia (Table VIII) shows no definite relation to the lime. The total varies but little from east to west and, except that it is lowest in the surface foot, shows no dependence upon the depth. In the most easterly two areas it is equal in amount to, or even higher than, the lime, but in the others it is much lower. The acid-soluble portion, except that it also is lowest in the surface foot, shows no dependence upon either the depth or the aridity of climate.

TABLE VII  
CARBON DIOXIDE IN THE FOOT-SECTIONS FROM DIFFERENT FIELDS IN THE  
WESTERLY AREAS

## WAUNETA

Depth Foot	Field I %	Field II %	Field III %	Field IV %	Field V %	Average %
3	.77	.17	.35	11	1.25	.53
4	1.77	1.68	1.14	1 73	2.00	1.66
5	1.77	1.01	1.76	1 90	2.11	1.71
6	2.00	1.18	1.56	1 44	2.04	1.64

## McCOOK.

2	.37	.09	.42	.09	1.05	.40
3	1.78	.71	2 04	1 54	3.45	1.90
4	1.95	1.83	2.57	1 84	3.05	2.25
5	1.93	1 99	2.16	1 74	2.50	2.06
6	1.99	1 72	2 11	1.61	2.35	1.96

## HOLDREGE.

4	.67	1.16	.45	.42	.33	.61
5	1.15	1.22	.71	1.06	.40	.91
6	1.17	1.19	.91	1.19	.62	1.01

Five-gram portions of the Wauneta and Lincoln samples were warmed with dilute (1 to 5) hydrochloric acid for 5 minutes in order to decompose the carbonates. The acid extract was then quickly filtered off, the residue washed and the lime and magnesia determined in the filtrate, in which would be found all of these two bases present in the soil in the form of carbonates, together with some derived from readily decomposable silicates (Table IX).

From the eastern, practically carbonate-free soils at Lincoln, the amount of magnesia dissolved is almost equal to that of the lime, while from the strongly effervescing subsoils at Wauneta more than twice as much lime as magnesia is dissolved. As there is more than enough readily soluble lime to account for all the carbon dioxide, evidently but a small portion of the carbonates, at most, consists of dolomite or magnesite.

## ALUMINA

The total alumina (Table X) is very uniformly distributed, it showing a maximum of 14.04, and a minimum of 10.88, with an average of 12.19 per cent. There is a slight decrease from east to west but this may be attributed to the leaching out of the calcium carbonate in the easterly

TABLE VIII  
MAGNESIA IN THE FOOT-SECTIONS

TOTAL							
Depth Foot	Wauneta %	McCook %	Holdrege %	Hastings %	Lincoln %	W. Water %	Average %
1	1.05	1.15	.90	.85	1.02	.88	.97
2	1.30	1.34	1.16	1.07	1.21	1.27	1.22
3	1.46	1.58	1.22	1.23	1.18	1.57	1.37
4	1.49	1.59	1.53	1.32	1.33	1.35	1.43
5	1.36	1.45	1.43	1.34	1.21	1.28	1.35
6	1.51	1.50	1.48	1.39	1.32	1.33	1.42
Average	1.36	1.43	1.29	1.20	1.21	1.28	1.29

ACID-SOLUBLE							
1	.83	.98	.81	.69	.76	.80	.81
2	.92	1.27	1.00	.80	.85	1.03	.98
3	.97	1.41	1.12	1.07	.93	1.12	1.10
4	.90	1.49	1.25	1.12	.88	1.01	1.11
5	1.04	1.41	1.32	1.16	.93	.94	1.13
6	1.01	1.43	1.32	1.23	.72	1.19	1.15
Average	.94	1.33	1.14	1.01	.84	1.01	1.05

ACID-INSOLUBLE							
1	.22	.17	.09	.16	.26	.08	.16
2	.38	.07	.16	.27	.36	.24	.24
3	.49	.17	.10	.16	.25	.45	.27
4	.59	.10	.28	.20	.45	.34	.32
5	.32	.04	.11	.18	.28	.34	.21
6	.49	.07	.16	.16	.60	.14	.27
Average	.42	.10	.15	.19	.37	.26	.24

TABLE IX  
RELATIVE AMOUNTS OF LIME AND MAGNESIA DISSOLVED BY DILUTE  
HYDROCHLORIC ACID FROM SEMI-ARID AND HUMID SOILS

Depth Foot	Wauneta		Lincoln	
	CaO %	MgO %	CaO %	MgO %
1	.42	.59	.36	.26
2	.48	.64	.39	.34
3	1.20	.69	.44	.38
4	2.35	.87	.48	.42
5	2.36	.91	.60	.45
6	2.32	.90	.49	.35
Average	1.52	.76	.46	.36

areas with a consequent concentration of the other constituents. In each area the first foot-sample contains less than the second but in the most westerly fields this difference is slight. This is to be attributed to the carrying of colloidal clay from the first foot into the lower levels. The acid-soluble portion is similar in the western four areas, but distinctly higher at Lincoln and Weeping Water. It also is lower in the first than in the second foot. The larger proportion of acid-soluble alumina in the Lincoln and Weeping Water areas may be attributed to the higher content of clay in these soils, the alumina in the latter being more readily soluble than that in the coarser fractions.

TABLE X  
ALUMINA IN THE FOOT-SECTIONS

TOTAL							
Depth Foot	Wauneta %	McCook %	Holdrege %	Hastings %	Lincoln %	W. Water %	Average %
1	11.32	11.33	10.88	11.05	11.43	11.57	11.26
2	11.80	11.73	12.75	12.52	14.04	12.81	12.61
3	11.87	11.66	12.08	13.31	13.42	12.91	12.54
4	11.91	11.43	11.54	12.18	13.00	13.20	12.21
5	11.01	11.76	11.26	12.45	13.26	13.00	12.12
6	11.17	11.86	12.76	12.80	12.94	12.76	12.38
Average	11.51	11.63	11.88	12.38	13.01	12.71	12.19
ACID-SOLUBLE							
1	6.94	6.80	5.72	5.84	8.34	8.30	7.00
2	7.73	8.16	7.67	7.99	11.61	10.43	8.93
3	7.91	7.64	8.14	8.41	10.28	10.93	8.89
4	7.41	7.81	7.54	7.90	10.25	9.70	8.44
5	7.07	7.84	6.96	7.40	10.22	9.48	8.16
6	6.97	7.80	7.14	7.16	10.00	9.52	8.10
Average	7.34	7.67	7.19	7.45	10.12	9.73	8.25
ACID-INSOLUBLE							
1	4.38	4.53	5.16	5.21	3.09	3.27	4.27
2	4.07	3.57	5.08	4.53	2.43	2.38	3.68
3	3.96	4.02	3.94	4.50	3.14	1.98	3.66
4	4.50	3.62	4.00	4.28	2.75	3.50	3.77
5	3.94	3.92	4.30	5.05	3.04	3.52	3.96
6	4.20	4.06	5.62	5.64	2.94	3.24	4.28
Average	4.17	3.96	4.69	4.93	2.89	2.98	3.93

Hall and Russell (10, p. 217) in an exhaustive study of a large number of English soils, have found close relationship between the clay content and the proportion of both potash and alumina dissolved by 48 hours' digestion on the water-bath with hydrochloric acid of 1.115 specific gravity. They included in clay only the particles below .002 mm. in diameter, while we include also those between .002 and .005 mm. They found the alumina commonly to amount to about one-third of the clay fraction and to ten times the potash. We find no relation between the potash and the

alumina (Tables XIX to XXIV); in the western areas the alumina equals the clay, and in the eastern it is more than half as high. If we had excluded the fraction between .002 and .005 mm. the ratio would have been still higher.

TABLE XI  
FERRIC OXIDE IN THE FOOT-SECTIONS

TOTAL							
Depth Foot	Wauneta %	McCook %	Holdrege %	Hastings %	Lincoln %	W. Water %	Average %
1	3.07	3.55	3.13	2.91	3.71	4.24	3.43
2	3.39	4.00	3.70	4.07	4.52	5.10	4.13
3	3.46	3.71	4.00	4.14	5.00	5.25	4.26
4	3.36	3.75	3.86	4.03	5.03	5.03	4.18
5	3.24	3.55	3.83	4.00	4.81	4.77	4.03
6	3.17	3.48	3.52	3.85	4.81	4.93	3.96
Average	3.28	3.67	3.67	3.83	4.65	4.89	4.00
ACID-SOLUBLE							
1	2.92	3.29	2.94	2.84	3.50	4.08	3.26
2	3.22	3.48	3.53	3.75	4.32	4.78	3.85
3	3.13	3.47	3.56	3.93	4.50	4.85	3.91
4	3.19	3.27	3.46	3.73	4.43	4.73	3.80
5	3.10	3.31	3.38	3.69	4.40	4.59	3.74
6	2.91	3.10	3.36	3.65	4.31	4.63	3.66
Average	3.08	3.32	3.37	3.60	4.24	4.61	3.70
ACID-INSOLUBLE							
1	.15	.26	.19	.07	.21	.16	.17
2	.17	.52	.17	.32	.20	.32	.28
3	.33	.24	.44	.21	.50	.40	.35
4	.17	.48	.40	.30	.60	.30	.38
5	.14	.24	.45	.31	.41	.18	.29
6	.26	.38	.16	.20	.50	.30	.30
Average	.20	.35	.30	.23	.40	.28	.29

### IRON

The iron is reported as ferric oxide (Table XI), no attempt having been made to determine the ferrous iron on account of the large amount of organic matter in the surface soils. The total amount is about 1 per cent higher in the Weeping Water and Lincoln areas than in those to the west. Like the alumina it is lower in the first than in the second foot, but the relative differences are much greater. While in most of the areas the ferric oxide and alumina show much similarity in distribution they do not reach their maxima in the same levels. The amounts of ferric oxide in the levels below the first foot are very similar, although the maximum usually is found in the third or fourth foot. At the end of 5 days' digestion with hydrochloric acid less than one-tenth of the iron remains undissolved, the proportion being independent of both depth and relative aridity. The subsoils from the Weeping Water and Lincoln areas, those in

which we find the highest proportion of iron, have a more distinctly yellow color. Without suggesting a cause for the higher iron content of these two areas it may be pointed out that they are underlaid by the highly ferruginous Kansan till and Dakota sandstone, neither of which underlies the loess of any of the other areas.

TABLE XII  
SILICA IN THE DIFFERENT FOOT-SECTIONS

Depth Foot	Wauneta %	McCook %	Holdrege %	Hastings %	Lincoln %	W. Water %	Average %
1	72.98	72.10	71.88	72.58	69.81	69.95	71.55
2	72.70	70.88	70.75	70.39	67.78	68.10	70.10
3	71.24	68.35	70.68	70.72	69.46	68.95	69.90
4	69.28	68.22	70.21	70.27	70.49	70.38	69.81
5	70.98	68.82	70.68	71.15	70.53	70.28	70.41
6	70.57	69.47	70.77	71.15	70.71	70.61	70.55
Average	71.29	69.64	70.83	71.04	69.80	69.66	70.38

TABLE XIII  
DIFFERENCES IN SILICA, ALUMINA AND FERRIC OXIDE BETWEEN THE FIRST AND SECOND FEET. IN THE FIRST FOOT THE SILICA IS HIGHER BUT THE ALUMINA AND THE FERRIC OXIDE ARE LOWER

	Wauneta %	McCook %	Holdrege %	Hastings %	Lincoln %	W. Water %
SiO <sub>2</sub>	28	1.22	1.13	2.19	2.03	1.85
Al <sub>2</sub> O <sub>3</sub>	— 48	— 40	— 1.57	— 1.47	— 2.61	— 1.24
Fe <sub>2</sub> O <sub>3</sub>	— 32	— 45	— 57	— 1.16	— 0.81	— 0.86

### SILICA

The silica (Table XII) is practically uniform throughout. There is no distinct difference between the eastern and the western soils, and but little between the amounts in the different foot-sections. As there is an average of about 5 per cent of carbonates in the soils from the two westerly areas the proportion of silica in the carbonate-free portion is really highest in these. There is slightly more in the first than in the second foot of each of the areas. In general the greater the difference in silica shown by the two levels the greater is that in alumina, but in the opposite direction. This will be evident from Table XIII. This relation is to be attributed to the colloidal clay and ferric hydrate having been carried down from the surface and deposited in the second foot, while the sand grains were left behind.

### SULPHUR

Sulphur is present in these soils probably only in the form of sulphates. The amount (Table XIV) is everywhere small and appears independent of both the depth and the relative aridity. In general a little more than half is soluble in hydrochloric acid. The differences in the amount of the insoluble portion are of no real significance. The experi-

mental errors in the determination of the small percentages of both total and acid-soluble sulphur, the data used to obtain the acid-insoluble portion, may either counterbalance one another, or, if in the same direction, double the error.

TABLE XIV  
SULPHURIC ACID IN THE FOOT-SECTIONS.

TOTAL							
Depth Foot	Wauneta %	McCook %	Holdrege %	Hastings %	Lincoln %	W. Water %	Average %
1	.09	.09	.08	.08	.07	.10	.08
2	.08	.09	.08	.09	.08	.10	.09
3	.06	.08	.05	.08	.10	.10	.08
4	.08	.09	.08	.06	.08	.06	.07
5	.08	.10	.09	.08	.06	.07	.08
6	.07	.09	.08	.06	.06	.05	.07
Average	.08	.09	.08	.07	.07	.08	.08

ACID SOLUBLE							
1	.03	.03	.05	.06	.05	.06	.05
2	.03	.03	.04	.04	.07	.07	.05
3	.03	.04	.04	.03	.04	.06	.04
4	.03	.04	.04	.04	.04	.04	.04
5	.03	.04	.06	.05	.05	.03	.04
6	.03	.05	.05	.05	.07	.03	.05
Average	.03	.04	.05	.05	.05	.05	.05

ACID-INSOLUBLE							
1	.06	.06	.03	.02	.02	.04	.04
2	.05	.06	.04	.05	.01	.03	.04
3	.03	.04	.01	.05	.06	.04	.04
4	.05	.05	.04	.02	.04	.02	.04
5	.05	.06	.03	.03	.01	.04	.04
6	.04	.04	.03	.01	.01	.02	.03
Average	.05	.05	.03	.04	.02	.03	.04

### BARYTA

The baryta, which is all insoluble (Table XV), is uniform in distribution. It is of interest that the amount of acid-insoluble  $\text{SO}_3$  corresponds to what would be required if the whole of it were present in combination as barium sulphate.

### MANGANESE

The manganese (Table XVI) is much higher in the eastern two areas than in those to the west, in this respect resembling the iron, but showing no relation to the depth.

The whole of it is soluble in hydrochloric acid.

## TITANIUM

The total titanium oxide (Table XVII), like the iron, is highest in the easterly two areas, while the acid-soluble portion shows no distinct differences. Neither bears any relation to the depth.

TABLE XV  
BARYTA IN THE FOOT-SECTIONS

## TOTAL

Depth Foot	Wauneta %	McCook %	Holdrege %	Hastings %	Lincoln %	W. Water %	Average %
1	.09	.07	.07	.07	.07	.06	.07
2	.06	.07	.07	.08	.07	.06	.07
3	.06	.07	.07	.08	.07	.09	.07
4	.06	.08	.09	.07	.07	.06	.07
5	.06	.08	.09	.07	.07	.09	.07
6	.05	.08	.06	.07	.08	.08	.07
Average	.06	.08	.08	.07	.07	.07	.07

TABLE XVI  
MANGANESE OXIDE IN THE FOOT-SECTIONS

Depth Foot	Wauneta %	McCook %	Holdrege %	Hastings %	Lincoln %	W. Water %	Average %
1	.05	.06	.06	.07	.10	.11	.08
2	.05	.06	.06	.07	.10	.12	.08
3	.05	.06	.06	.07	.11	.12	.08
4	.05	.06	.06	.07	.12	.12	.08
5	.05	.06	.06	.07	.12	.12	.08
6	.05	.06	.06	.07	.12	.15	.08
Average	.05	.06	.06	.07	.11	.12	.08

The small amounts of calcium carbonate in the eastern areas may be explained on the assumption that what was originally present has been leached out, but a difference in climate would fail to account for the distinctly larger amounts of iron, titanium and manganese found in the areas overlying the Kansan drift.

## WATER-SOLUBLE MATERIAL

In all the area foot-samples we determined the total amount of water-soluble material, including both organic and inorganic, by agitation with carbon dioxide-free water, filtration through a Chamberland-Pasteur filter, evaporation on the water-bath and subsequent drying at 105° C. It varied from 0.05 to 0.12 per cent, the amount present not being related to the relative aridity.



TABLE XVII  
TITANIUM OXIDE IN THE FOOT-SECTIONS

TOTAL							
Depth Foot	Wauneta %	McCook %	Holdrege %	Hastings %	Lincoln %	W. Water %	Average %
1	1.08	.98	.98	1.03	1.24	1.24	1.09
2	1.10	.98	.98	1.04	1.25	1.25	1.10
3	1.10	.98	.99	1.03	1.25	1.25	1.10
4	1.10	.98	.98	1.03	1.25	1.29	1.10
5	1.10	.98	.96	1.03	1.14	1.30	1.08
6	1.10	.98	.98	1.03	1.16	1.29	1.09
Average	1.10	.98	.98	1.03	1.21	1.27	1.09

ACID-SOLUBLE							
1	.26	.43	.21	.32	.16	.24	.27
2	.29	.24	.29	.17	.20	.22	.23
3	.30	.23	.24	.22	.18	.23	.23
4	.22	.19	.30	.10	.22	.31	.22
5	.20	.17	.27	.22	.08	.28	.20
6	.26	.18	.28	.13	.18	.28	.22
Average	.25	.24	.26	.19	.17	.26	.23

ACID-INSOLUBLE							
1	.82	.55	.77	.71	1.08	1.00	.82
2	.81	.74	.69	.87	1.05	1.03	.86
3	.80	.75	.75	.81	1.07	1.02	.87
4	.88	.79	.68	.93	1.03	.98	.88
5	.90	.81	.69	.81	1.06	1.02	.88
6	.84	.80	.70	.90	.98	1.01	.87
Average	.85	.74	.71	.84	1.04	1.01	.86

#### REACTION TO LITMUS

The acidity, as this term is commonly used in regard to soils, decreases from east to west. When tested with litmus the eastern soils showed a neutral and the western a slightly alkaline reaction, the intensity in the case of the latter being greater in the lower levels.

#### ACID-INSOLUBLE MATTER

The proportion which remains after digesting 5 days with hydrochloric acid and subsequently igniting (Table XVIII-A) is highest at Wauneta, lowest at Weeping Water and intermediate and similar in the intervening areas, except that it is higher in the first than in the second foot and shows no dependence upon the depth. However, such a presentation of the data may be somewhat misleading, as the amount of insoluble matter thus found is greatly affected by the proportion of both the organic matter and the carbonates. The relation of the insoluble matter to the non-volatile, carbonate-free portion of the samples will give us a much better idea of the variations in the sum of the silica and the insoluble silicates. This is shown in Table XVIII-B. In preparing this we cal-

culated the amount of the carbonate on the assumption that the whole of the carbon dioxide is present in the form of the calcium salt. Thus in the case of the fifth foot from Wauneta, in which there is 2.75 per cent of volatile matter and 1.78 per cent of carbon dioxide, the insoluble matter, 79.88 per cent, is derived from 93.21 per cent of non-volatile, carbonate-free material, and so forms 85.71 per cent of this. The highest amount is found at Wauneta and the lowest at Weeping Water. The Lincoln area closely resembles the latter, while Hastings, Holdrege and McCook show an intermediate composition. In all the areas the proportion of the insoluble material in the first foot is higher than in any of the underlying sections. The latter are similar. This would indicate that the leaching has somewhat affected the silicates in the surface foot, but not in the lower levels.

TABLE XVIII  
ACID-INSOLUBLE MATTER

A.—IN MOISTURE FREE SAMPLES

Depth Foot	Wauneta %	McCook %	Holdrege %	Hastings %	Lincoln %	W. Water %	Average %
1	82.28	79.98	80.77	81.11	76.65	76.07	79.48
2	81.67	78.17	78.60	78.38	74.82	74.47	77.68
3	80.77	76.04	78.63	78.22	76.61	75.50	77.63
4	79.18	76.57	78.75	78.89	77.51	76.64	77.92
5	79.88	77.19	79.43	79.65	78.01	77.82	78.66
6	79.97	77.67	79.50	79.84	78.96	78.01	78.99
Average	80.62	77.60	79.28	79.35	77.09	76.42	78.39

B.—IN NON-VOLATILE, CARBONATE-FREE PORTIONS

1	86.84	84.90	86.96	86.53	83.73	84.13	85.51
2	85.24	82.66	82.88	82.94	81.26	80.26	82.54
3	85.39	82.93	82.57	82.21	80.97	79.77	82.31
4	85.37	83.91	83.50	82.87	81.04	80.21	82.82
5	85.70	83.96	84.15	83.18	81.55	81.32	83.31
6	85.79	84.18	84.12	83.73	82.32	81.28	83.57
Average	85.72	83.76	84.03	83.58	81.81	81.16	83.34

For convenience of reference and comparison the data are summarized in Tables XIX to XXVI. The data on the volatile matter are those reported by Alway and McDole (2, p. 232) and those on potash, soda and phosphoric acid reported by Alway and Isham, (1, p. 301).

COMPARISON WITH CHERNOZEM SOILS

The *mechanical composition* of the Chernozem soils is very uniform from the surface downward and shows no definite relation to the depth (14, p. 300); this is true also of the Nebraska loess. In the case of the latter there is a little less clay in the first than in the second foot but the available data on the Chernozem do not permit of a comparison on this point.

TABLE XIX  
COMPOSITION OF SOILS FROM WAUNETA AREA

## COMPLETE ANALYSIS

	1st Foot %	2d Foot %	3d Foot %	4th Foot %	5th Foot %	6th Foot %	Average %
SiO <sub>2</sub>	72.98	72.70	71.24	69.28	70.98	70.57	71.29
Al <sub>2</sub> O <sub>3</sub>	11.32	11.80	11.87	11.91	11.01	11.17	11.51
Fe <sub>2</sub> O <sub>3</sub>	3.07	3.39	3.46	3.36	3.24	3.17	3.28
MnO	.05	.05	.05	.05	.05	.05	.05
MgO	1.05	1.30	1.46	1.49	1.36	1.51	1.36
CaO	1.67	1.69	2.72	3.93	3.91	3.94	2.98
Na <sub>2</sub> O	1.41	1.43	1.34	1.42	1.45	1.48	1.42
K <sub>2</sub> O	2.63	2.68	2.70	2.65	2.67	2.75	2.68
TiO <sub>2</sub>	1.08	1.10	1.10	1.10	1.10	1.10	1.10
P <sub>2</sub> O <sub>5</sub>	.12	.13	.12	.15	.15	.14	.13
CO <sub>2</sub>	.09	.07	.59	1.67	1.78	1.68	.98
SO <sub>3</sub>	.09	.08	.06	.08	.08	.07	.08
BaO	.09	.06	.06	.06	.06	.05	.06
Volatile matter	5.05	4.03	4.08	3.47	2.75	2.97	3.72
Total	100.70	100.51	100.85	100.62	100.59	100.65	100.64

## DIGESTION WITH HYDROCHLORIC ACID

Insoluble	82.28	81.67	80.77	79.18	79.88	79.97	80.62
Al <sub>2</sub> O <sub>3</sub>	6.94	7.73	7.91	7.41	7.07	6.97	7.34
Fe <sub>2</sub> O <sub>3</sub>	2.92	3.22	3.13	3.19	3.10	2.91	3.08
MnO	.06	.06	.06	.06	.06	.06	.06
MgO	.83	.92	.97	.90	1.04	1.01	.94
CaO	1.10	1.15	1.81	3.06	3.04	3.43	2.26
Na <sub>2</sub> O	.32	.37	.43	.33	.53	.43	.40
K <sub>2</sub> O	.96	1.17	1.06	1.14	1.16	1.16	1.11
P <sub>2</sub> O <sub>5</sub>	.12	.12	.10	.13	.13	.12	.12
TiO <sub>2</sub>	.26	.29	.30	.22	.20	.26	.25
CO <sub>2</sub>	.09	.07	.59	1.67	1.78	1.68	.98
SO <sub>3</sub>	.03	.03	.03	.03	.03	.03	.03
Volatile matter	5.05	4.03	4.08	3.47	2.75	2.97	3.72
Total	100.96	100.83	101.24	100.79	100.77	101.00	100.91

## ACID-INSOLUBLE PORTION

SiO <sub>2</sub>	72.98	72.70	71.24	69.28	70.98	70.57	71.29
Al <sub>2</sub> O <sub>3</sub>	4.38	4.07	3.96	4.50	3.94	4.20	4.17
Fe <sub>2</sub> O <sub>3</sub>	.15	.17	.33	.27	.14	.26	.22
MnO	.00	.00	.00	.00	.00	.00	.00
MgO	.22	.38	.49	.59	.32	.49	.42
CaO	.57	.54	.91	.87	.87	.51	.72
Na <sub>2</sub> O	1.09	1.06	.91	1.09	.92	1.05	1.02
K <sub>2</sub> O	1.67	1.51	1.64	1.51	1.51	1.59	1.57
P <sub>2</sub> O <sub>5</sub>	.00	.01	.02	.02	.02	.02	.01
TiO <sub>2</sub>	.82	.81	.80	.88	.90	.84	.85
SO <sub>3</sub>	.06	.05	.03	.05	.05	.04	.05
BaO	.09	.06	.06	.06	.06	.06	.06
Total	82.03	81.36	80.39	79.22	79.81	79.63	80.38

TABLE XX  
COMPOSITION OF SOILS FROM McCOOK AREA

## COMPLETE ANALYSIS

	1st Foot %	2d Foot %	3d Foot %	4th Foot %	5th Foot %	6th Foot %	Average %
SiO <sub>2</sub>	72.10	70.88	68.35	68.22	68.82	69.47	69.64
Al <sub>2</sub> O <sub>3</sub>	11.33	11.73	11.66	11.43	11.76	11.86	11.63
Fe <sub>2</sub> O <sub>3</sub>	3.55	4.00	3.71	3.75	3.55	3.48	3.67
MnO	.06	.06	.06	.06	.06	.06	.06
MgO	1.15	1.34	1.58	1.59	1.45	1.50	1.43
CaO	1.40	1.80	3.59	3.91	3.54	3.44	2.95
Na <sub>2</sub> O	1.50	1.49	1.40	1.36	1.51	1.50	1.46
K <sub>2</sub> O	2.51	2.49	2.50	2.55	2.63	2.60	2.55
TiO <sub>2</sub>	.98	.98	.98	.98	.98	.98	.98
P <sub>2</sub> O <sub>5</sub>	.13	.12	.12	.12	.13	.13	.12
CO <sub>2</sub>	.02	.40	2.02	2.34	2.11	2.08	1.49
SO <sub>2</sub>	.09	.09	.08	.09	.10	.09	.09
BaO	.07	.07	.07	.08	.08	.08	.08
Volatile matter	5.71	4.52	3.74	3.44	3.28	3.00	3.95
Total	100.60	99.97	99.86	99.92	100.00	100.27	100.10

## DIGESTION WITH HYDROCHLORIC ACID

	79.98	78.17	76.04	76.57	77.19	77.67	77.60
Insoluble	79.98	78.17	76.04	76.57	77.19	77.67	77.60
Al <sub>2</sub> O <sub>3</sub>	6.80	8.16	7.64	7.81	7.84	7.80	7.67
Fe <sub>2</sub> O <sub>3</sub>	3.29	3.48	3.47	3.27	3.31	3.10	3.32
MnO	.06	.06	.06	.06	.06	.06	.06
MgO	.98	1.27	1.41	1.49	1.41	1.43	1.33
CaO	.88	1.38	3.03	3.33	3.04	2.84	2.42
Na <sub>2</sub> O	.47	.41	.49	.45	.41	.38	.43
K <sub>2</sub> O	1.15	1.23	1.27	1.22	1.21	1.22	1.22
P <sub>2</sub> O <sub>5</sub>	.12	.11	.11	.10	.10	.11	.11
TiO <sub>2</sub>	.43	.24	.28	.19	.17	.18	.24
CO <sub>2</sub>	.02	.40	2.02	2.34	2.11	2.08	1.49
SO <sub>2</sub>	.03	.03	.04	.04	.04	.05	.04
Volatile matter	5.70	4.52	3.74	3.44	3.28	3.00	3.95
Total	99.91	99.46	99.55	100.31	100.17	99.92	99.88

## ACID-INSOLUBLE PORTION

	72.10	70.88	68.35	68.22	68.82	69.47	69.64
SiO <sub>2</sub>	72.10	70.88	68.35	68.22	68.82	69.47	69.64
Al <sub>2</sub> O <sub>3</sub>	4.53	3.57	4.02	3.62	3.92	4.06	3.96
Fe <sub>2</sub> O <sub>3</sub>	.26	.52	.24	.48	.24	.38	.35
MnO	.00	.00	.00	.00	.00	.00	.00
MgO	.17	.07	.17	.10	.04	.07	.10
CaO	.52	.42	.56	.58	.50	.60	.53
Na <sub>2</sub> O	1.03	1.08	.91	.91	1.10	1.12	1.03
K <sub>2</sub> O	1.36	1.26	1.23	1.33	1.42	1.38	1.33
P <sub>2</sub> O <sub>5</sub>	.01	.01	.01	.02	.03	.02	.01
TiO <sub>2</sub>	.55	.74	.75	.79	.81	.80	.74
SO <sub>2</sub>	.06	.06	.04	.05	.06	.04	.05
BaO	.07	.07	.07	.08	.08	.08	.07
Total	80.66	78.68	76.35	76.18	77.02	78.02	77.81

TABLE XXI  
COMPOSITION OF SOILS FROM HOLDREGE AREA

## COMPLETE ANALYSIS

	1st Foot %	2d Foot %	3d Foot %	4th Foot %	5th Foot %	6th Foot %	Average %
SiO <sub>2</sub>	71.88	70.75	70.68	70.21	70.68	70.77	70.83
Al <sub>2</sub> O <sub>3</sub>	10.88	12.75	12.08	11.54	11.26	12.76	11.88
Fe <sub>2</sub> O <sub>3</sub>	3.13	3.70	4.00	3.86	3.83	3.52	3.67
MnO	.06	.06	.06	.06	.06	.06	.06
MgO	.90	1.16	1.22	1.53	1.43	1.48	1.29
CaO	1.18	1.33	1.60	2.15	2.30	2.19	1.79
Na <sub>2</sub> O	1.50	1.38	1.40	1.44	1.57	1.49	1.46
K <sub>2</sub> O	2.40	2.46	2.56	2.67	2.64	2.66	2.36
TiO <sub>2</sub>	.98	.98	.99	.98	.96	.98	.98
P <sub>2</sub> O <sub>5</sub>	.14	.11	.13	.15	.13	.11	.13
CO <sub>2</sub>	.01	.03	.13	.75	1.00	1.05	.49
SO <sub>3</sub>	.08	.08	.05	.08	.09	.08	.08
BaO	.07	.07	.07	.09	.09	.06	.08
Volatile matter	7.10	5.10	4.48	3.98	3.34	3.10	4.52
Total	100.31	99.96	99.45	99.49	99.38	100.31	99.82

## DIGESTION WITH HYDROCHLORIC ACID

	80.77	78.60	78.63	78.75	79.43	79.50	79.28
Insoluble	5.72	7.67	8.14	7.54	6.96	7.14	7.19
Fe <sub>2</sub> O <sub>3</sub>	2.94	3.53	3.56	3.46	3.38	3.36	3.37
MnO	.05	.05	.05	.05	.05	.05	.05
MgO	.81	1.00	1.12	1.25	1.32	1.32	1.14
CaO	.74	.86	.97	1.69	1.84	1.84	1.32
Na <sub>2</sub> O	.32	.42	.50	.50	.45	.47	.44
K <sub>2</sub> O	1.13	1.35	1.33	1.36	1.32	1.32	1.30
P <sub>2</sub> O <sub>5</sub>	.11	.10	.13	.14	.13	.09	.12
TiO <sub>2</sub>	.21	.29	.24	.30	.27	.28	.26
CO <sub>2</sub>	.01	.03	.13	.75	1.00	1.05	.49
SO <sub>3</sub>	.05	.04	.04	.04	.06	.05	.05
Volatile matter	7.10	5.10	4.48	3.98	3.34	3.10	4.52
Total	99.96	95.04	99.32	99.81	99.55	99.57	99.53

## ACID-INSOLUBLE PORTION

	71.88	70.75	70.68	70.21	70.68	70.77	70.83
SiO <sub>2</sub>	5.16	5.08	3.94	4.00	4.30	5.62	4.69
Al <sub>2</sub> O <sub>3</sub>	.19	.17	.44	.40	.45	.16	.30
Fe <sub>2</sub> O <sub>3</sub>	.01	.01	.01	.01	.01	.01	.01
MnO	.09	.16	.10	.28	.11	.16	.15
CaO	.44	.47	.63	.46	.46	.35	.47
Na <sub>2</sub> O	1.18	.96	.90	.94	1.12	1.02	1.02
K <sub>2</sub> O	1.27	1.11	1.23	1.31	1.32	1.34	1.26
P <sub>2</sub> O <sub>5</sub>	.03	.01	.00	.01	.00	.02	.01
TiO <sub>2</sub>	.77	.69	.75	.68	.69	.70	.71
SO <sub>3</sub>	.03	.04	.01	.04	.03	.03	.03
BaO	.07	.07	.07	.09	.09	.06	.07
Total	81.12	79.52	78.76	78.43	79.26	80.24	79.55

TABLE XXII  
COMPOSITION OF SOILS FROM HASTINGS AREA

## COMPLETE ANALYSIS

	1st Foot %	2d Foot %	3d Foot %	4th Foot %	5th Foot %	6th Foot %	Average %
SiO <sub>2</sub>	72.58	70.39	70.72	70.27	71.15	71.15	71.04
Al <sub>2</sub> O <sub>3</sub>	11.05	12.52	13.31	12.18	12.45	12.80	12.39
Fe <sub>2</sub> O <sub>3</sub>	2.91	4.07	4.14	4.03	4.00	3.85	3.83
MnO	.07	.07	.07	.07	.07	.07	.07
MgO	.85	1.07	1.23	1.32	1.34	1.39	1.20
CaO	1.13	1.16	1.45	1.75	1.80	1.72	1.50
Na <sub>2</sub> O	1.48	1.36	1.36	1.59	1.47	1.54	1.47
K <sub>2</sub> O	2.49	2.45	2.51	2.56	2.67	2.65	2.55
TiO <sub>2</sub>	1.03	1.04	1.03	1.03	1.03	1.03	1.03
P <sub>2</sub> O <sub>5</sub>	.11	.11	.12	.11	.13	.15	.12
CO <sub>2</sub>	.01	.02	.10	.36	.38	.41	.21
SO <sub>3</sub>	.08	.09	.08	.06	.08	.06	.07
BaO	.07	.08	.08	.07	.07	.07	.07
Volatile matter	6.25	5.45	4.63	4.00	3.83	3.71	4.64
Total	100.11	99.88	100.83	99.40	100.47	100.60	100.18

## DIGESTION WITH HYDROCHLORIC ACID

	81.11	78.38	78.22	78.89	79.65	79.84	79.35
Insoluble	81.11	78.38	78.22	78.89	79.65	79.84	79.35
Al <sub>2</sub> O <sub>3</sub>	5.84	7.99	8.41	7.90	7.40	7.16	7.45
Fe <sub>2</sub> O <sub>3</sub>	2.84	3.75	3.93	3.73	3.69	3.65	3.60
MnO	.05	.05	.06	.04	.04	.04	.05
MgO	.69	.80	1.07	1.12	1.16	1.23	1.01
CaO	.68	.74	.97	1.22	1.33	1.36	1.05
Na <sub>2</sub> O	.48	.45	.46	.54	.46	.42	.47
K <sub>2</sub> O	1.15	1.42	1.46	1.36	1.38	1.35	1.35
P <sub>2</sub> O <sub>5</sub>	.10	.11	.12	.10	.13	.13	.11
TiO <sub>2</sub>	.32	.17	.22	.10	.22	.13	.19
CO <sub>2</sub>	.01	.02	.10	.36	.38	.41	.21
SO <sub>3</sub>	.06	.04	.03	.04	.05	.05	.05
Volatile matter	6.25	5.45	4.63	4.00	3.83	3.71	4.64
Total	99.58	99.37	99.68	99.40	99.72	99.48	99.53

## ACID-INSOLUBLE PORTION

	72.58	70.39	70.72	70.27	71.15	71.15	71.04
SiO <sub>2</sub>	72.58	70.39	70.72	70.27	71.15	71.15	71.04
Al <sub>2</sub> O <sub>3</sub>	5.21	4.53	4.90	4.28	5.05	5.64	4.93
Fe <sub>2</sub> O <sub>3</sub>	.07	.32	.21	.30	.31	.20	.23
MnO	.02	.02	.01	.03	.03	.03	.02
MgO	.16	.27	.16	.20	.18	.16	.19
CaO	.45	.42	.48	.53	.47	.36	.45
Na <sub>2</sub> O	1.00	.91	.90	1.05	1.01	1.12	1.00
K <sub>2</sub> O	1.34	1.03	1.05	1.20	1.29	1.30	1.20
P <sub>2</sub> O <sub>5</sub>	.01	.00	.00	.01	.00	.02	.01
TiO <sub>2</sub>	.71	.87	.81	.93	.81	.90	.84
SO <sub>3</sub>	.02	.05	.05	.02	.03	.01	.03
BaO	.07	.08	.08	.07	.07	.07	.07
Total	81.64	78.88	79.37	78.89	80.40	80.92	80.01

TABLE XXIII  
COMPOSITION OF SOILS FROM LINCOLN AREA

## COMPLETE ANALYSIS

	1st Foot %	2d Foot %	3d Foot %	4th Foot %	5th Foot %	6th Foot %	Average %
SiO <sub>2</sub>	69.81	67.78	69.46	70.49	70.53	70.71	69.80
Al <sub>2</sub> O <sub>3</sub>	11.43	14.04	13.42	13.00	13.26	12.94	13.01
Fe <sub>2</sub> O <sub>3</sub>	3.71	4.52	5.00	5.03	4.81	4.81	4.65
MnO	.10	.10	.11	.12	.12	.12	.11
MgO	1.02	1.21	1.18	1.33	1.21	1.32	1.21
CaO	.72	1.00	1.21	1.21	1.39	1.31	1.14
Na <sub>2</sub> O	.96	.94	1.06	1.14	1.21	1.18	1.08
K <sub>2</sub> O	2.46	2.47	2.51	2.54	2.52	2.53	2.50
TiO <sub>2</sub>	1.24	1.25	1.25	1.25	1.14	1.16	1.21
P <sub>2</sub> O <sub>5</sub>	.13	.14	.16	.17	.19	.17	.16
CO <sub>2</sub>	.01	.02	.02	.06	.12	.08	.05
SO <sub>3</sub>	.07	.08	.10	.08	.06	.06	.07
BaO	.07	.07	.07	.07	.07	.08	.07
Volatile matter	8.44	6.68	5.25	4.22	4.07	3.90	5.43
Total	100.17	100.30	100.80	100.71	100.70	100.37	100.49

## DIGESTION WITH HYDROCHLORIC ACID

Insoluble	76.65	74.82	76.61	77.51	78.01	78.96	77.09
Al <sub>2</sub> O <sub>3</sub>	8.34	11.61	10.28	10.25	10.22	10.00	10.12
Fe <sub>2</sub> O <sub>3</sub>	3.50	4.32	4.50	4.43	4.40	4.31	4.24
MnO	.09	.09	.10	.09	.09	.09	.09
MgO	.76	.85	.93	.88	.93	.72	.84
CaO	.61	.64	.75	.90	1.10	.96	.83
Na <sub>2</sub> O	.39	.45	.43	.51	.48	.46	.45
K <sub>2</sub> O	1.09	1.14	1.16	1.26	1.29	1.27	1.20
P <sub>2</sub> O <sub>5</sub>	.11	.12	.11	.14	.13	.14	.12
TiO <sub>2</sub>	.16	.20	.18	.22	.08	.18	.17
CO <sub>2</sub>	.01	.02	.02	.06	.12	.08	.05
SO <sub>3</sub>	.05	.07	.04	.04	.05	.07	.05
Volatile matter	8.44	6.68	5.25	4.22	4.07	3.90	5.43
Total	100.20	101.01	100.36	100.51	100.97	101.14	100.68

## ACID-INSOLUBLE PORTION

SiO <sub>2</sub>	69.81	67.78	69.46	70.49	70.53	70.71	69.80
Al <sub>2</sub> O <sub>3</sub>	3.09	2.43	3.14	2.75	3.04	2.94	2.89
Fe <sub>2</sub> O <sub>3</sub>	.21	.20	.50	.60	.41	.50	.40
MnO	.02	.02	.01	.03	.03	.03	.02
MgO	.26	.36	.25	.45	.28	.60	.37
CaO	.11	.36	.46	.31	.29	.35	.31
Na <sub>2</sub> O	.57	.49	.63	.63	.73	.72	.63
K <sub>2</sub> O	1.37	1.33	1.35	1.28	1.23	1.26	1.30
P <sub>2</sub> O <sub>5</sub>	.02	.02	.05	.03	.06	.03	.03
TiO <sub>2</sub>	1.08	1.05	1.07	1.03	1.06	.98	1.04
SO <sub>3</sub>	.02	.01	.06	.04	.01	.01	.02
BaO	.07	.07	.07	.07	.07	.08	.07
Total	76.63	74.12	77.05	77.71	77.74	78.21	76.88

TABLE XXIV  
COMPOSITION OF SOILS FROM WEEPING WATER AREA

## COMPLETE ANALYSIS

	1st Foot %	2d Foot %	3d Foot %	4th Foot %	5th Foot %	6th Foot %	Average %
SiO <sub>2</sub>	69.65	68.10	68.95	70.38	70.28	70.61	69.66
Al <sub>2</sub> O <sub>3</sub>	11.57	12.81	12.91	13.20	13.00	12.76	12.71
Fe <sub>2</sub> O <sub>3</sub>	4.24	5.10	5.25	5.03	4.77	4.93	4.89
MnO	.11	.12	.12	.12	.12	.15	.12
MgO	.88	1.27	1.57	1.35	1.28	1.33	1.28
CaO	.89	.95	1.37	1.08	1.08	1.18	1.09
Na <sub>2</sub> O	1.05	.99	1.04	1.29	1.27	1.37	1.17
K <sub>2</sub> O	2.46	2.38	2.42	2.37	2.45	2.42	2.42
TiO <sub>2</sub>	1.24	1.25	1.25	1.29	1.30	1.29	1.27
P <sub>2</sub> O <sub>5</sub>	.13	.12	.13	.16	.18	.17	.15
CO <sub>2</sub>	.01	.01	.05	.00	.02	.01	.02
SO <sub>2</sub>	.10	.10	.10	.06	.07	.05	.08
BaO	.06	.06	.09	.06	.09	.08	.07
Volatile matter	8.43	7.17	5.24	4.46	4.27	3.99	5.59
Total	100.82	100.43	100.49	100.85	100.18	100.34	100.52

## DIGESTION WITH HYDROCHLORIC ACID

Insoluble	76.07	74.47	75.50	76.64	77.82	78.01	76.42
Al <sub>2</sub> O <sub>3</sub>	8.30	10.43	10.93	9.70	9.48	9.52	9.73
Fe <sub>2</sub> O <sub>3</sub>	4.08	4.78	4.85	4.73	4.59	4.63	4.61
MnO	.10	.11	.11	.12	.12	.13	.11
MgO	.80	1.03	1.12	1.01	.94	1.19	1.01
CaO	.68	.71	.76	.78	.78	.99	.78
Na <sub>2</sub> O	.23	.26	.33	.29	.37	.33	.30
K <sub>2</sub> O	1.25	1.42	1.43	1.37	1.38	1.37	1.37
P <sub>2</sub> O <sub>5</sub>	.10	.10	.11	.16	.16	.17	.13
TiO <sub>2</sub>	.24	.22	.23	.31	.28	.28	.26
CO <sub>2</sub>	.01	.01	.05	.00	.02	.01	.02
SO <sub>2</sub>	.06	.07	.06	.04	.03	.03	.05
Volatile matter	8.43	7.17	5.24	4.46	4.27	3.99	5.59
Total	100.35	100.80	100.72	99.61	100.24	100.65	100.38

## ACID-INSOLUBLE PORTION

SiO <sub>2</sub>	69.65	68.10	68.95	70.38	70.28	70.61	69.66
Al <sub>2</sub> O <sub>3</sub>	3.27	2.38	1.98	3.50	3.52	3.24	2.98
Fe <sub>2</sub> O <sub>3</sub>	.16	.32	.40	.30	.18	.30	.28
MnO	.01	.01	.01	.00	.00	.02	.01
MgO	.08	.24	.45	.34	.34	.14	.26
CaO	.21	.24	.61	.30	.30	.19	.31
Na <sub>2</sub> O	.82	.73	.71	1.00	.90	1.04	.87
K <sub>2</sub> O	1.21	.96	.99	1.00	1.07	1.05	1.05
P <sub>2</sub> O <sub>5</sub>	.03	.02	.02	.00	.02	.00	.02
TiO <sub>2</sub>	1.00	1.03	1.02	.98	1.02	1.01	1.01
SO <sub>2</sub>	.04	.03	.04	.02	.04	.02	.03
BaO	.06	.06	.09	.06	.09	.08	.07
Total	76.54	74.12	75.27	77.88	77.76	77.70	76.55



TABLE XXV  
AVERAGE COMPOSITION OF THE SOIL FROM THE DIFFERENT LEVELS

## COMPLETE ANALYSIS

	1st Foot %	2d Foot %	3d Foot %	4th Foot %	5th Foot %	6th Foot %	Average %
SiO <sub>2</sub>	71.55	70.10	69.90	69.81	70.41	70.55	70.38
Al <sub>2</sub> O <sub>3</sub>	11.26	12.61	12.54	12.21	12.12	12.38	12.19
Fe <sub>2</sub> O <sub>3</sub>	3.43	4.13	4.26	4.18	4.03	3.96	4.00
MnO	.08	.08	.08	.08	.08	.08	.08
MgO	.97	1.22	1.37	1.43	1.35	1.42	1.29
CaO	1.16	1.32	1.99	2.34	2.34	2.30	1.91
Na <sub>2</sub> O	1.32	1.27	1.27	1.37	1.41	1.42	1.34
K <sub>2</sub> O	2.49	2.49	2.53	2.56	2.60	2.60	2.54
TiO <sub>2</sub>	1.09	1.10	1.10	1.10	1.08	1.09	1.09
P <sub>2</sub> O <sub>5</sub>	.13	.12	.13	.14	.15	.14	.13
CO <sub>2</sub>	.02	.09	.48	.87	.90	.88	.54
SO <sub>2</sub>	.08	.09	.08	.07	.08	.07	.08
BaO	.07	.07	.07	.07	.07	.07	.07
Volatile matter	6.82	5.49	4.57	3.92	3.59	3.44	4.64
Total	100.47	100.18	100.37	100.15	100.21	100.40	100.28

## DIGESTION WITH HYDROCHLORIC ACID

	79.48	77.68	77.63	77.92	78.66	78.99	78.39
Insoluble	79.48	77.68	77.63	77.92	78.66	78.99	78.39
Al <sub>2</sub> O <sub>3</sub>	7.00	8.93	8.89	8.44	8.16	8.10	8.25
Fe <sub>2</sub> O <sub>3</sub>	3.26	3.85	3.91	3.80	3.74	3.66	3.70
MnO	.07	.07	.07	.07	.07	.07	.07
MgO	.81	.98	1.10	1.11	1.13	1.15	1.05
CaO	.78	.91	1.38	1.83	1.85	1.90	1.44
Na <sub>2</sub> O	.37	.39	.44	.44	.45	.41	.42
K <sub>2</sub> O	1.12	1.29	1.28	1.28	1.29	1.28	1.26
P <sub>2</sub> O <sub>5</sub>	.11	.11	.11	.13	.13	.13	.12
TiO <sub>2</sub>	.27	.23	.23	.22	.20	.22	.23
CO <sub>2</sub>	.02	.09	.48	.87	.90	.88	.54
SO <sub>2</sub>	.05	.05	.04	.04	.04	.05	.05
Volatile matter	6.82	5.49	4.57	3.92	3.59	3.44	4.64
Total	100.16	100.07	100.13	100.07	100.21	100.28	100.16

## ACID-INSOLUBLE PORTION

	71.55	70.10	69.90	69.81	70.41	70.55	70.36
SiO <sub>2</sub>	71.55	70.10	69.90	69.81	70.41	70.55	70.36
Al <sub>2</sub> O <sub>3</sub>	4.27	3.68	3.66	3.77	3.96	4.28	3.93
Fe <sub>2</sub> O <sub>3</sub>	.17	.28	.35	.38	.29	.30	.29
MnO	.01	.01	.01	.01	.01	.02	.01
MgO	.16	.24	.27	.32	.21	.27	.24
CaO	.38	.41	.61	.51	.48	.39	.46
Na <sub>2</sub> O	.95	.88	.83	.93	.96	1.01	.92
K <sub>2</sub> O	1.37	1.20	1.25	1.28	1.31	1.32	1.28
P <sub>2</sub> O <sub>5</sub>	.02	.01	.02	.01	.02	.01	.02
TiO <sub>2</sub>	.82	.86	.87	.88	.88	.87	.86
SO <sub>2</sub>	.03	.04	.04	.04	.04	.02	.04
BaO	.07	.07	.07	.07	.07	.07	.07
Total	79.80	77.78	77.88	78.01	78.64	79.11	78.48

TABLE XXVI

AVERAGE COMPOSITION OF THE SIX FEET OF SOIL FROM THE DIFFERENT AREAS

## COMPLETE ANALYSIS

	Wauneta %	McCook %	Holdrege %	Hastings %	Lincoln %	W.Water %	Average %
SiO <sub>2</sub>	71.29	69.64	70.83	71.04	69.80	69.66	70.38
Al <sub>2</sub> O <sub>3</sub>	11.51	11.63	11.88	12.38	13.01	12.71	12.19
Fe <sub>2</sub> O <sub>3</sub>	3.28	3.67	3.67	3.83	4.65	4.89	4.00
MnO	.05	.06	.06	.07	.11	.12	.08
MgO	1.36	1.43	1.29	1.20	1.21	1.28	1.29
CaO	2.98	2.95	1.79	1.50	1.14	1.09	1.91
Na <sub>2</sub> O	1.42	1.46	1.46	1.47	1.08	1.17	1.34
K <sub>2</sub> O	2.68	2.55	2.56	2.55	2.50	2.42	2.54
TiO <sub>2</sub>	1.10	.98	.98	1.03	1.21	1.27	1.09
P <sub>2</sub> O <sub>5</sub>	.13	.12	.13	.12	.16	.15	.13
CO <sub>2</sub>	.98	1.49	.49	.21	.05	.02	.54
SO <sub>3</sub>	.08	.09	.08	.07	.07	.08	.08
BaO	.06	.08	.08	.07	.07	.07	.07
Volatile matter	3.72	3.95	4.52	4.64	5.43	5.59	4.64
Total	100.64	100.10	99.82	100.18	100.49	100.52	100.28

## DIGESTION WITH HYDROCHLORIC ACID

Insoluble	80.62	77.60	79.28	79.35	77.09	76.42	78.39
Al <sub>2</sub> O <sub>3</sub>	7.34	7.67	7.19	7.45	10.12	9.73	8.25
Fe <sub>2</sub> O <sub>3</sub>	3.08	3.32	3.37	3.60	4.24	4.61	3.70
MnO	.06	.06	.05	.05	.09	.11	.07
MgO	.94	1.33	1.14	1.01	.84	1.01	1.05
CaO	2.26	2.42	1.32	1.05	.83	.78	1.44
Na <sub>2</sub> O	.40	.43	.44	.47	.45	.30	.42
K <sub>2</sub> O	1.11	1.22	1.30	1.35	1.20	1.37	1.26
P <sub>2</sub> O <sub>5</sub>	.12	.11	.12	.11	.12	.13	.12
TiO <sub>2</sub>	.25	.24	.26	.19	.17	.26	.23
CO <sub>2</sub>	.98	1.49	.49	.21	.05	.02	.54
SO <sub>3</sub>	.03	.04	.05	.05	.05	.05	.05
Volatile matter	3.72	3.95	4.52	4.64	5.43	5.59	4.64
Total	100.91	99.88	99.53	99.53	100.68	100.38	100.16

## ACID-INSOLUBLE PORTION

SiO <sub>2</sub>	71.29	69.64	70.83	71.04	69.80	69.66	70.36
Al <sub>2</sub> O <sub>3</sub>	4.17	3.96	4.69	4.93	2.89	2.98	3.93
Fe <sub>2</sub> O <sub>3</sub>	.20	.35	.30	.23	.40	.28	.29
MnO	.00	.00	.01	.02	.02	.01	.01
MgO	.42	.10	.15	.19	.37	.26	.24
CaO	.72	.53	.47	.45	.31	.31	.46
Na <sub>2</sub> O	1.10	1.03	1.02	1.00	.63	.87	.92
K <sub>2</sub> O	1.49	1.33	1.26	1.20	1.30	1.05	1.28
P <sub>2</sub> O <sub>5</sub>	.02	.01	.01	.01	.03	.02	.02
TiO <sub>2</sub>	.85	.74	.71	.84	1.04	1.01	.86
SO <sub>3</sub>	.05	.05	.03	.03	.02	.03	.04
BaO	.06	.08	.08	.07	.07	.07	.07
Total	80.38	77.82	79.56	80.01	76.88	76.55	78.48

The Chernozem soils, excluding those with appreciable amounts of carbonates, contain approximately 1.5 to 2.0 per cent of *lime*, of which usually not less than one per cent is acid-soluble (14, p. 326). The loess soils show a quite similar composition, although containing somewhat smaller amounts in the eastern areas.

In the Chernozem the *magnesia* (14, p. 326), both the total and the zeolitic portion, is usually somewhat lower than the lime. In this respect the loess soils are similar except that in the eastern two areas both the total and the acid-soluble magnesia are as high as the lime.

The total *alumina* in the Chernozem soils (14, p. 326) lies between approximately 10 and 15 per cent, about half being dissolved by a 10-hour digestion on the water-bath with 10 per cent hydrochloric acid. In the total amount the loess soils are similar but the data on the acid-soluble portions are not comparable on account of the differences in the strength of acid used and the time of digestion. The amount of alumina dissolved increases steadily during the five days' digestion, which would make it probable that the two types of soils are similar in their content of zeolitic alumina.

The *iron oxide* of the Chernozem soils varies from 2 to 5 per cent and this is almost completely acid-soluble (14, p. 396); in the loess samples we found from 3 to 5 per cent, nearly all acid-soluble.

The data on sulphur in Chernozem soils Kossowitsch (14, p. 327) considers untrustworthy. It has been reported, expressed as  $\text{SO}_3$ , to vary from 0.10 to 0.30 per cent. In none of the loess samples did we find more than 0.10 per cent.

The *water-soluble material* in the Chernozem soils Kossowitsch (14, p. 327) reports as generally less than 0.10 per cent (*ca.* 0.08 per cent) of which about half consists of organic substances. The loess soils show a similar amount.

In their *reaction* toward litmus the loess soils of Nebraska resemble the Chernozem soils of which the typical representatives (14, p. 322) possess a neutral, or those of the drier portions even a faintly alkaline, reaction; by the chestnut-colored Chernozem soils the alkaline reaction is most common, while the soils of the more humid areas may show a faintly acid reaction.

Comparisons of the potash, soda and phosphoric acid have previously been made (1, p. 313).

On the basis of the mechanical composition and of the chemical composition of the inorganic portion, including the zeolitic portion, the loess soils of the Nebraska portion of the Transition Region are very similar to the Chernozem soils of Russia.

## COMPARISON WITH ARID SOILS

Hilgard, who first recognized the characteristic differences between humid and arid soils, has recently (12, p. 424) compared the average composition of 313 arid soils from the Pacific slope with that of 466 humid soils from the states south of the Ohio River. All determinations of the inorganic portions were made by digestion with hydrochloric acid of 1.115 specific gravity for 5 days. Hence our data on the acid-soluble portion reported in the above tables are strictly comparable with his. In Table XXVII we give Hilgard's data in comparison with ours. In the case of the latter we employ only those from the first foot samples, and use the average from adjacent areas so that we have the humid eastern portion, the distinctly semi-arid western, and finally the intermediate portion including the Hastings and Holdrege areas.

TABLE XXVII

COMPARISON OF THE TRANSITION SOILS WITH ARID AND HUMID SOILS  
REPORTED BY HILGARD

	Average of 313 %	Semi-arid Western areas %	Intermediate Transition areas %	Humid Eastern areas %	Average of 466 %
Insoluble	77.82	81.13	80.94	77.57	88.24
K <sub>2</sub> O	.73	1.05	1.14	1.12	.22
Na <sub>2</sub> O	.26	.40	.40	.31	.09
CaO	1.36	.99	.71	.65	.11
MgO	1.41	.90	.75	.78	.23
Mn <sub>2</sub> O <sub>4</sub>	.06	.07	.09	.14	.13
Fe <sub>2</sub> O <sub>3</sub>	5.75	3.10	2.89	3.79	3.13
Al <sub>2</sub> O <sub>3</sub>	7.89	6.87	5.78	8.32	4.30
P <sub>2</sub> O <sub>5</sub>	.12	.12	.11	.11	.11
SO <sub>3</sub>	.04	.03	.05	.05	.05
CO <sub>2</sub>	1.32	.05	.01	.01	...
Volatile	4.94	5.37	6.67	8.43	3.64

In lime and magnesia as well as in the amount of insoluble matter all the Transition soils resemble the arid soils. The same is true of potash and soda. In carbon dioxide content all resemble the humid, the carbonates, even in the distinctly semi-arid areas, having been leached out of the surface foot. In manganese the soils from the most humid eastern two areas resemble the humid soils reported by Hilgard, while those from the other four resemble the arid. This difference in manganese content which Hilgard considers too frequent to be accidental (12, p. 392) is, as he suggests, due to some obscure cause. In the other inorganic constituents no general marked difference is observed between arid and humid soils.

Thus it is evident that the surface soils from all six areas, even the most humid, resemble the strictly arid soils reported by Hilgard.

## SUMMARY

The loess soils of the Nebraska portion of the Transition Region consist chiefly of very fine sand and silt which together constitute from 77 to 95 per cent of the soil mass, the remainder being chiefly clay. As we pass from east to west, the clay decreases and the relative proportions of the silt and the very fine sand change, the former decreasing and the latter increasing.

The mechanical composition shows no distinct relation to the depth except that the clay content is lower in the first than in the second foot.

The values for the hygroscopic coefficients calculated from the mechanical analyses by the formula proposed by Briggs and Shantz agree satisfactorily with those obtained by direct determination in the case of only the samples with the smallest proportion of very fine sand. However, by altering the values assigned the sands, a formula has been obtained which is applicable to the soils from all the areas.

The samples were subjected to both a complete rock analysis and to 5-day digestion with hydrochloric acid of 1.115 specific gravity. The carbon dioxide, which is present chiefly in calcium carbonate, shows greater variations than any other constituent; while low in the first two feet of all the areas, the amount in the subsoil increases markedly as we pass from east to west. The lime varies widely, both the total and the acid-soluble portion, being three times as high in the western subsoils as in the eastern. The content of magnesia shows no definite relation to that of the lime, in the eastern areas it being as high but in the western much lower; it is independent of the aridity and, except that it is lowest in the surface foot, also of the depth. The total alumina is very uniformly distributed but in all the areas shows a minimum in the surface foot. The acid-soluble portion is similar in the western four areas, but markedly higher in the eastern two; like the total it is lower in the first than in the second foot. It shows no definite relation to either the clay or the acid-soluble potash. The iron, manganese and titanium are distinctly higher in the eastern two than in the other four areas. Almost the whole of the iron is acid-soluble; like the alumina it shows a minimum in the surface foot. The whole of the manganese is acid-soluble, but only a small part of the titanium. The silica is very uniformly distributed but, in contrast to the alumina, is in each area slightly higher in the first than in the second foot. Sulphur and baryta show no dependence upon either depth or aridity. About half of the former is acid-soluble, but none of the latter. To litmus the samples are all neutral or very slightly alkaline.

The acid-insoluble matter shows no definite relation to the aridity and, except that it is higher in the first than in the second foot, none to the depth. The proportion of acid-insoluble material in the non-volatile, car-

bonate-free portion of the soil is highest in the surface foot and similar in the lower levels, as though leaching had affected the silicates of only the first foot.

In mechanical composition these loess soils show the same characteristics as the Russian Chernozem. Also, in the chemical composition of the inorganic portion, both the total and the acid-soluble, in so far as the available data permit of comparisons, there is a very marked similarity.

A comparison with the average composition of arid and humid soils, as reported by Hilgard, shows that, except in the proportions of manganese, the first foot samples of the loess soils from the most humid areas studied resemble the arid soils as much as do those from the distinctly semi-arid western areas. In the case of this one constituent the soils from the eastern areas resemble those from the humid regions reported by Hilgard. In carbonate content the subsoils from the western and intermediate areas resemble arid subsoils and those from the eastern areas the humid soils.

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# STUDIES ON THE DECOMPOSITION OF CELLULOSE IN SOILS<sup>1</sup>

By

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## INTRODUCTION

The discovery and comprehension of the biological and chemical forces relating to the decomposition of the carbohydrate materials in soils is unquestionably necessary to the solution of many problems in soil fertility and crop production. A large percentage of the carbon content of the plant residue is found as a constituent of the celluloses. These compounds, because of their refractory nature, must first be attacked by a special group of organisms. An accurate knowledge of the cultural and biochemical characteristics of the organisms involved in the transformation of cellulose into less refractory compounds is, therefore, obviously of the greatest importance.

Extensive investigations during the last few years have shown that the decomposition of cellulose is by no means limited to the bacteria of soils. The filamentous fungi possessing this power are very numerous and many species are exceedingly active agents in the destruction of cellulose. In the humid soils of the East the filamentous fungi are perhaps of greater importance than bacteria in the destruction of cellulose, while in the semi-arid soils of the West the reverse is apparently true. Several species of *Actinomyces* are also known to have the power of dissolving cellulose and because of their general distribution, these organisms are undoubtedly a factor in the destruction of cellulose in soils. This paper deals, for the most part, with investigations of cellulose-dissolving bacteria.

## CULTURE MEDIA

Methods for the preparation of cellulose agar and other suitable culture media for the study of cellulose-dissolving bacteria have been discussed, at some length, in earlier publications by Kellerman and McBeth (29), McBeth and Scales (43), Löhnis and Lochhead (40), Kellerman,

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<sup>1</sup> Paper No. 16, Citrus Experiment Station, College of Agriculture, University of California, Riverside, California.

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A bibliography of the literature relating to cellulose destruction is included and reference is made by numbers to "literature cited" (p. 481).



McBeth, Scales, and Smith (30), and Scales (71). The cellulose agar prepared as described in the above mentioned publications has given very satisfactory results not only with cellulose-dissolving bacteria, but also with filamentous fungi. The medium also appears to be well adapted to the study of cellulose destruction by species of *Actinomyces*. We have frequently observed colonies of *Actinomyces* which dissolve the cellulose very rapidly in the cellulose agar, forming a clear enzymic zone about the colony which furnishes unmistakable evidence of the cellulose-dissolving power of the organism. Krainsky (36) in his recent studies of the *Actinomyces*, has reported the cellulose agar plate method as unsatisfactory for determining the cellulose-dissolving power of these organisms. However, he was able to demonstrate the cellulose-dissolving power of several species of *Actinomyces* by the use of paper pulp or strips of paper on silica jelly, and also by means of cellulose hydrate prepared by the zinc chloride method. The reason for the failure of Krainsky to secure satisfactory results with the cellulose agar plate method is not clear. However, since these organisms grew luxuriantly upon the cellulose agar prepared by us and dissolved the cellulose very rapidly, it would seem that the unsatisfactory results reported by Krainsky may be due to certain inattention to details in the preparation of the cellulose precipitate. In order to secure a uniformly fine amorphous precipitate, it is necessary to carry out the operations with considerable care. It is believed that much of the difficulty experienced in the preparation of precipitated cellulose is caused by precipitating in solutions that are too concentrated. If either the copper-ammonium-cellulose solution or the acid used in precipitating the cellulose is too concentrated, a product is frequently secured which is not only difficult to wash, but is very unsatisfactory as a culture medium. A very uniform and satisfactory amorphous precipitate can be secured by adhering strictly to the following method which is a slight modification of the method originally proposed.

1. Pour 1 liter of ammonium hydroxide, sp. gr. 0.90, into a glass-stoppered bottle; add 250 c.c. of distilled water and 75 gm. of pure copper carbonate; shake the solution vigorously until all the copper is dissolved. (From 10 to 15 minutes is ordinarily required.)

2. To the copper-ammonium solution add 15 gm. of high grade, sheet filter paper; shake vigorously at intervals of 10 minutes for one-half hour. Examine the solution carefully to see that the paper is completely dissolved. If any particles of paper remain in the solution, the shaking must be continued until the solution is perfectly clear.

Dilute 250 c.c. of the ammonium-copper-cellulose solution to 10 liters with tap water; add slowly with frequent shaking, a weak hydrochloric acid solution prepared by adding 500 c.c. of concentrated acid to 10 liters of

tap water. Continue the addition of the acid until the blue color disappears; add a slight excess of acid, shake thoroughly and allow to stand a few minutes. The finely precipitated cellulose will rise to the top, due to the large quantity of free hydrogen liberated in the precipitation process. Shake the solution vigorously at intervals of a few minutes to dislodge the hydrogen. As soon as the free hydrogen has escaped the cellulose will settle rapidly.

3. Wash through repeated changes of water until free from copper and chlorine. After the washing is complete, bring the cellulose in the solution up to 0.5 per cent, by allowing to settle a few days and siphoning off the clear solution or by evaporating. Add the nutrient salts desired together with 1 per cent of thoroughly washed agar; heat in autoclave or boil until the agar is dissolved; tube and sterilize in the usual way.

#### ACTION OF THE CELLULOSE-DISSOLVING BACTERIA STUDIED ON THE CELLULOSE OF PLANT TISSUES

While the preparation of cellulose agar from precipitated cellulose as described above has proven quite satisfactory for the isolation and study of organisms which dissolve typical cellulose, such as is found in filter paper or in cotton fiber, it does not make possible a study of the action of the organisms on the celluloses in plant tissues such as are ordinarily added to the soil, as stubble, roots, green manure, etc. Since the term "cellulose" connotes a group of substances rather than a single chemical compound, it seems important that methods be devised which will make possible a comparative study of the action of the cellulose-dissolving organisms isolated from the soil, upon the cellulose of different plants and also of the same plants at different stages of maturity. In the young plant cells the walls contain almost pure cellulose, but as the plant develops the cellulose originally formed is altered by the addition to it of various secondary products known as encrusting substances. The nature and properties of the resulting fiber depends, of course, upon the nature of the substances deposited.

Since many of the cellulose-dissolving organisms attack not only the celluloses, but many other plant substances such as the starches, sugars, and proteins, it is necessary in studying the action of these organisms on the cellulose of different plant tissues, other than that of cotton fiber, to separate the cellulose from the other compounds with which it is more or less closely associated in the plant. It is also important that the purified cellulose be separated into very fine particles such as will permit the preparation of a satisfactory cellulose agar. Finely divided pure cellulose suitable for the preparation of cellulose agar may be prepared from plant substances as follows:

1. Grind a quantity of the dry plant substance to a flour and sift through bolting cloth to remove all coarse material.
2. Boil 50 gm. of the sifted flour in a 2 per cent potassium hydrate solution for one-half hour; pour into a large bottle or carboy and wash through repeated changes of water until free from potassium.
3. Expose the washed material to the action of chlorine at ordinary temperatures for one-half hour. Wash as before until the chlorine is removed.
4. Subject to a second alkaline hydrolysis by boiling with 2 per cent caustic soda for one-half hour. Wash until the solution is no longer alkaline.

The cellulose is thus isolated in a very pure state, and if the grinding of the plant material has been sufficiently fine, the finely divided cellulose prepared in this way is quite as satisfactory for the preparation of cellulose agar as that prepared from filter paper by the ordinary method.

In the present work it has not been possible to make an extensive study of the decomposition of the celluloses in different plant substances. However, it has been demonstrated that the cellulose-dissolving bacteria isolated from soils by means of the cellulose agar plate method, have the power of dissolving the cellulose of alfalfa. Twenty-five species of cellulose-dissolving bacteria were plated to cellulose agar containing pure cellulose from the alfalfa plant and in every instance the cellulose was dissolved as readily as that prepared from filter paper by the ordinary method.

#### DISCUSSION OF GENERAL CHARACTERISTICS OF CELLULOSE-DISSOLVING BACTERIA

The author's exhaustive studies of a large number of soils from widely separated regions have shown that there are numerous species of bacteria which have the power to destroy cellulose. All of the forms studied are rod-shaped organisms varying in length from .8 to 3.50  $\mu$ . Involution forms have been observed for only three species. Five species have been found to produce spores. Twenty-seven of the thirty-six species isolated are motile. The arrangement of the flagella on the motile forms shows that seven species belong to the genus *Pseudomonas* and twenty to the genus *Bacillus*. All species stain readily with the aniline dyes. All are facultative in nature, but invariably develop most rapidly under aerobic conditions. With some species, the development under anaerobic conditions is very slow. All species grow well from 20° to 37.5° C., and some forms have been found to develop at temperatures as high as 45° C., but much more slowly than at the lower temperatures. The optimum temperature for most species seems to lie between 28° to 33° C.

With two exceptions, the cellulose-destroying bacteria form more or less growth upon ordinary culture media such as beef gelatin, beef agar,

etc. Of the thirty-four species which grow upon gelatin, nineteen liquefy the gelatin. Many forms produce a growth upon beef agar and potato agar slopes in 24 hours. A few species grow quite luxuriantly upon potato cylinders, but in most cases no growth or only a scant growth is produced, even when the cultures are held in a moist chamber for 30 days. Twenty-nine species produce an acid reaction and three an alkaline reaction in litmus milk. Four species do not change the reaction of litmus milk. The milk is coagulated or digested by only six species.

The destruction of cellulose can be secured in nutrient solutions containing ammonium sulphate, potassium nitrate, peptone, casein, or asparagin as the source of nitrogen. Peptone appears to give the best results for the largest number of organisms, while casein is least satisfactory for many forms. No destruction of cellulose has been secured without the addition of combined nitrogen to the nutrient solution. This would seem to indicate that the cellulose-dissolving organisms do not draw freely upon the free nitrogen of the air for their nitrogen supply. This hypothesis is further strengthened by the behavior of the organisms in dextrose solutions. When dextrose is added to nutrient solutions containing combined nitrogen, many of the cellulose-dissolving organisms vigorously attack the dextrose; but when the nutrient solution is carefully freed from combined nitrogen the dextrose is attacked very slowly and little or no fixation of nitrogen is secured.

No gas is formed by any of the species in cellulose or other carbohydrate broths. The quantity of acid produced in carbohydrate broths is fairly constant for the species, but quite variable for different species. With dextrose, lactose, maltose, saccharose, and starch the quantity of acid produced in 12 days at 30° C. usually lies between 1 and 2 per cent on Fuller's Scale. The amount of acidity in the mannite and glycerine solutions is very generally less than 1 per cent, and in many cases no acidity is produced in these solutions. Two species cause no change in the reaction of any of the carbohydrate broths. *B. rossicus* gave an alkaline reaction in all the broths, while *Ps. effusa* gave an alkaline reaction in the lactose and saccharose broths. The alkaline reaction is probably due to the formation of ammonia from the peptone in the solution, the ammonia produced being more than sufficient to neutralize any acid formed. In Dunham's solution fourteen species produce ammonia, while twenty forms produce a compound which gives typical reactions for nitrites with the Griess' reagent and also with the starch-iodide and the diphenylamine solutions. There seems to be no reason for concluding that the substance is not nitrite except that nitrite formation has been thought to be restricted to a particular group of organisms which do not grow upon ordinary media. The quantity of nitrite formed by the cellulose-dissolving forms is small; in most instances not more than one

part per million of nitrogen as nitrite is produced. However, the formation of this small amount is constant and is, therefore, of considerable value as a diagnostic feature.

Since many species produce nitrites in Dunham's solution, it is obvious that erroneous conclusions might be drawn from the use of a nitrate broth containing peptone. Peptone has therefore been left out of the nitrate broth used in studying the nitrate reducing power of these organisms, and a small quantity of starch added to furnish the necessary carbon. In this broth many of the species reduce nitrates to nitrites, but only four forms reduce nitrates to ammonia.

#### THE OCCURRENCE AND ACTIVITY OF CELLULOSE-DISSOLVING BACTERIA IN SOUTHERN CALIFORNIA SOILS

Examinations of 69 soils of southern California for cellulose-dissolving bacteria indicate that these soils contain numerous species of bacteria which have the power of dissolving cellulose. All of the soils examined were found to contain one or more active cellulose-destroying forms and most of the species isolated were found in two or more soils from widely separated districts. One of the most active forms (*B. imminutus*) was isolated from ten of the sixty-nine soils examined. From the southern California soils studied fifteen new species of cellulose-dissolving bacteria have been isolated and described. In addition to the new species found, seven species previously isolated from other soils have been identified. The distribution of the cellulose-dissolving bacteria found in the southern California soils is shown in Table I.

It is well known that a very rapid destruction of cellulose occurs in many citrus soils of southern California. The question naturally arises whether the rapid destruction of cellulose in these soils is due to the presence of unusually active cellulose-destroying organisms or to favorable conditions which make possible a very rapid multiplication of the cellulose-dissolving organisms present. From the studies made, it is evident that the soils are abundantly supplied with active cellulose-destroying bacteria. Moreover, some of the most active forms appear to have a very wide distribution in the soils of southern California. However, with the possible exception of *B. imminutus* the cellulose-destroying bacteria found in southern California soils, when placed under standard conditions, appear to be no more active agents in the destruction of cellulose than the organisms isolated from the humid regions of the United States. In any explanation of the rapid destruction of cellulose in these soils we must take into consideration the activity of filamentous fungi and possibly the Actinomyces. The cellulose-destroying fungi are unquestionably less numerous and less active in the semi-arid soils of southern California than in the humid soils of the eastern part of the United States. The same is apparently true of the cellulose-destroying species of Actinomyces.



The writer's extensive studies of the cultural characteristics of cellulose-destroying organisms has shown that a rapid destruction of cellulose occurs only when the culture medium is thoroughly aerated and contains an abundant supply of available nitrogen. It is also essential that fairly high temperatures be maintained. The thorough cultivation given most citrus soils in southern California insures thorough aeration. The surface soil to which the organic matter is usually added is generally well supplied with available nitrogen. The soil temperature even during the winter months is seldom below that at which a rapid multiplication of the cellulose-dissolving organisms takes place. In view of the above stated conditions, it would seem that the very rapid destruction of cellulose in these soils is probably due more to the very favorable cultural and climatic conditions which make possible the rapid multiplication of the cellulose-dissolving organisms in these soils.

#### NEW SPECIES OF CELLULOSE-DISSOLVING BACTERIA

It is obvious that an adequate knowledge of cellulose decomposition in soils must be based upon a clear understanding of the character of the cellulose-dissolving micro-flora of soils. This knowledge can be obtained only by an arrangement of the organisms studied in a logical system of classification such as will make possible a comparative study of the forms described. In the establishment of the points of differentiation upon which separation may be based, there are of course many possible methods of procedure varying according to the points of resemblance which are selected as important.

In working out the description of new species of cellulose-dissolving bacteria, an attempt has been made to bring out the individual characteristics as concisely as possible. Many of the data called for by the card of the Society of American Bacteriologists seem to have little significance in the separation of members of this group. Moreover, in the isolation and classification of this group of organisms it has been found necessary to prepare several new varieties or culture media which are of especial importance in the classification of the cellulose-dissolving organisms, but would probably be of little importance in the classification of ordinary saprophytic bacteria in soils. So far as we are able to determine none of the cellulose-dissolving organisms isolated have been previously described as saprophytic forms. In view of the above stated conditions and the fact that the power to dissolve cellulose forms a definite basis for the group, we believe that the classification of the cellulose-dissolving organisms can be most satisfactorily accomplished by the employment of only those media which are of especial importance in differentiating the members of this particular group and by using only those characters which remain constant through several sets of cultures.

*Bacillus albidus*, n. sp.

SOURCE: Soil from Tustin, California.

## I. MORPHOLOGY.

1. Vegetative cells: Average dimensions  $1 \times .004 \mu$ .
2. Endospores: None observed.
3. Flagella: 1 to 3 in number; 3 to  $5 \mu$  in length.
4. Staining reactions: Gram negative. Stain readily with the aniline dyes.

## II. CULTURAL CHARACTERISTICS.

5. Agar strokes, 5 days.

*Beef Agar*: Scant, white, spreading growth.

*Potato agar*: Abundant, white to grayish white growth, spreading over the entire slope.

*Peptone starch agar*: Moderate, white to gray white growth.

6. *Potato cylinders*: No growth in 30 days.

7. *Gelatin stab*: Scant growth at surface and perceptible growth along the track of the needle; in 5 days. No liquefaction in 30 days.

8. *Beef broth*, 5 days. Not clouded

9. *Litmus milk*: Reddened in 7 days, neither coagulated nor digested in 30 days.

10. Plate cultures.

*Ammonia cellulose agar*, 15 days

Form: Colonies at the immediate surface of the medium are round, those located a little beneath the surface are irregularly round.

Size: 8 to 12 mm

Enzymic zone. Clearing all within colony after 15 days. After 30 days the colonies show an enzymic zone of 1 to 2 mm.

Elevation: Saucer shaped.

Chromogenesis: Entire colony is vitreous with the exception of a thin, white rim.

Internal structure: Indeterminate

Edge: Entire to undulate

*Peptone starch agar*, 5 days.

Form: Colonies at the immediate surface are round, those slightly below the surface are irregularly round.

Size: 2 to 3 mm.

Enzymic zone: 1 to 1.5 mm.

Elevation: Slightly convex.

Chromogenesis: White to light grayish white.

Internal structure: Coarsely granular; granules often arranged in clumps.

Edge: Entire to undulate.

*Beef agar*, 5 days.

Form: Round.

Size: 1 to 1.50 mm.

Elevation: Convex.

Consistency: Soft; colonies from 10 to 15 days old become brittle.

Chromogenesis: By reflected light the colonies are light grayish white. By transmitted light they appear as semi-transparent glistening drops.

Internal structure: Granular.

Edge: Entire.



*Potato agar*, 5 days.

Form: Round.

Size: 2 to 3 mm.

Elevation: Convex.

Consistency: Soft, colonies from 15 to 20 days old become brittle.

Chromogenesis: Semi-transparent white, with pearl-like luster.

Internal structure: Granular.

11. *Filter paper broths*, 15 days. The paper is reduced to a thin, filmy grayish white mass which readily breaks up on slight agitation. The paper is readily attacked in solutions supplied with ammonium sulphate or peptone; but is much slower in solutions containing potassium nitrate or casein as the source of nitrogen.

### III. BIOCHEMICAL FEATURES.

12. *Dunham's solution*, 10 days: No ammonia produced; no nitrite produced.
13. *Starch nitrate broth*, 10 days: No ammonia produced; no nitrite produced.
14. *Peptone nitrite solution*, 10 days: No indol produced.
15. *Carbohydrate broths*, 12 days: No gas produced. Per cent of acidity (Fuller's Scale) with: Dextrose, .50; Lactose, .20; Saccharose, .10; Maltose, .10; Glycerine, .10; Mannite, .10; Starch, .10.

### *Bacillus almus*, n. sp.

SOURCE: Soil from Arlington, California; Bonito, California, and Pasadena, California.

#### I. MORPHOLOGY.

1. Vegetative cells: Average dimensions  $1.2 \times .5 \mu$ .
2. Endospores: None observed.
3. Flagella: 1 to 5 in number; 3 to  $4 \mu$  in length.
4. Staining reactions: Gram negative. Stains readily with the aniline dyes.

#### II. CULTURAL CHARACTERISTICS.

5. Agar strokes, 5 days.  
*Beef agar*: Scant, white to grayish white growth. On slopes from 10 to 15 days old the growth becomes yellowish white.  
*Potato agar*: Moderate, glistening, grayish white growth. After 10 days, the growth becomes yellowish.  
*Peptone starch agar*: Moderate, glistening, grayish white growth, which becomes yellowish on old slopes.
6. *Potato cylinders*: No growth in 30 days.
7. *Gelatin stab*: Scant growth at surface and along track of the needle, in 5 days. No liquefaction in 30 days.
8. *Beef broth*, 5 days. Lightly clouded.
9. *Litmus milk*: Reddened in 6 days; neither coagulated nor digested in 30 days.
10. Plate cultures.  
*Ammonia cellulose agar*, 15 days.  
 Form: Round.  
 Size: 4 to 6 mm. in 15 days; 6 to 8 mm. in 30 days.  
 Enzymic zone: 1 to 1.5 mm. in 15 days; in 25 days 3 to 4 mm.  
 Elevation: Saucer shaped.

**Chromogenesis:** Semi-transparent, grayish white after 15 days; older colonies become yellowish white with a narrow grayish white rim.

**Internal structure:** Colony is made up of fine loosely arranged granules. The rim of the older colonies is composed of large granules compactly arranged.

**Edge:** Entire.

*Peptone cellulose agar, 15 days.*

**Form:** Round or irregularly round.

**Size:** 5 to 7 mm. in 15 days; in 25 days colonies frequently attain a diameter of 20 mm.

**Enzymic zone:** 2 mm. in 15 days; 2.5 to 3.5 mm. in 30 days.

**Elevation:** Saucer shaped.

**Chromogenesis:** Central portion of colony 2 to 3 mm. in diameter is semi-transparent, grayish white; outer portion of colony is vitreous. The colony is usually surrounded by a narrow white rim.

**Internal structure:** Central portion of colony is granular; structure of the vitreous portion is indeterminate.

**Edge:** Entire to undulate

*Peptone starch agar, 5 days*

**Form:** Irregular Those colonies at the immediate surface are round or nearly round, but those beneath the surface and the bottom colonies are quite irregular in outline.

**Size:** 2 to 3 mm.

**Enzymic zone:** 3 to 4 mm.

**Elevation:** Flat or very slightly convex.

**Chromogenesis:** The surface colonies show a small white nucleus, the remainder of the colony grayish white. The imbedded and bottom colonies are grayish to grayish white.

**Internal structure:** Granular.

**Edge:** Lacerate

*Beef agar, 5 days*

**Form:** Round.

**Size:** Surface colonies 1 to 1.5 mm.; bottom colonies 2 to 3 mm.

**Elevation:** Convex.

**Consistency:** Colony is soft during the first 10 days, after which it becomes brittle.

**Chromogenesis:** By reflected light the colonies are white to light grayish white. By transmitted light they are translucent light, smoky brown.

**Structure:** Granular

**Edge:** Entire.

*Potato agar, 5 days.*

**Form:** Round.

**Size:** Surface colonies, 1 to 2 mm.; bottom colonies, 2 to 3 mm.

**Elevation:** Pulvinate.

**Consistency:** Butyrous after 5 days; somewhat viscous after 10 days.

**Chromogenesis:** Glistening, yellowish to grayish white.

**Internal structure:** Granular.

**Edge:** Entire.

11. *Filter paper broths*, 15 days. Paper reduced to a loose, felt-like mass which retains the pure white color of the paper. The structure of the paper has been entirely destroyed, as can be easily demonstrated by the slight agitation of the solution. The decomposition of the paper was less rapid with casein or potassium nitrate as the source of nitrogen than with peptone or ammonium sulphate.

### III. BIOCHEMICAL FEATURES.

12. *Dunham's solution*, 10 days: No ammonia produced; no nitrite produced.
13. *Starch nitrate broth*, 10 days: No ammonia produced; no nitrite produced.
14. *Peptone nitrite solution*, 10 days: No indol produced.
15. *Carbohydrate broths*, 12 days: No gas produced. Per cent of acidity (Fuller's Scale) with: Dextrose, 1.30; Lactose, .80; Saccharose, 1.00; Maltose, 1.20; Glycerine, .40; Mannite, .00; Starch, .60.

### *Bacillus concitatus*, n. sp.

SOURCE: Soil from Barstow, California; Covina, California; Riverside, California.

#### I. MORPHOLOGY.

1. Vegetative cells: Average dimensions,  $1.2 \times .5 \mu$ .
2. Endospores: None observed.
3. Flagella: 1 to 3 in number; 3 to  $4 \mu$  in length.
4. Staining reactions: Gram negative. Stains readily with the aniline dyes.

#### II. CULTURAL CHARACTERISTICS.

5. Agar strokes, 5 days.  
*Beef agar*: Abundant, flat, moist, yellowish white.  
*Potato agar*: Abundant, raised, moist, glistening, grayish white; old cultures become somewhat yellowish white.  
*Peptone starch agar*: Abundant, raised, frequently somewhat rugose, grayish white.
6. *Potato cylinders*: No growth in 30 days.
7. *Gelatin stab*: Moderate growth at surface and along stab in 5 days; slight napiform liquefaction after 30 days.
8. *Beef broth*, 5 days: Heavily clouded.
9. *Litmus milk*: Reddened in 4 days; no curdling or digestion apparent after 30 days.
10. Plate cultures.  
*Ammonia cellulose agar*, 15 days.  
 Form: Surface colonies are round or irregularly round; bottom colonies spread out into irregular somewhat amoeboid growths.  
 Size: Surface colonies are from 1 to 5 mm.; bottom colonies frequently attain a diameter of 15 mm.  
 Enzymic zone: Surface colonies, 1 to 1.5 mm.; bottom colonies sometimes show no enzymic zone, but the colony is always more transparent than the surrounding medium, showing that some of the cellulose within the colony has been dissolved.  
 Elevation: Flat or slightly depressed.  
 Chromogenesis: Many of the colonies are almost pure white, while others show very thin brownish rings.  
 Internal structure: Brownish rings coarsely granular; remainder of colony finely granular.  
 Edge: Entire.

*Peptone cellulose agar, 15 days.*

Form: Surface colonies round; bottom colonies irregularly round.

Size: Surface colonies, 1 to 2 mm.; bottom colonies 12 to 15 mm.

Enzymic zone: Surface colonies, 2 to 2.5 mm.; bottom colonies, 1 mm. or less.

Elevation: Flat or very slightly convex.

Chromogenesis: Central portion of colony opaque white; outer portion, semi-transparent grayish white. Brownish rings sometimes apparent.

Internal structure: Central portion of colony coarsely granular, remainder of colony finely granular.

Edge: Usually entire, but some colonies throw out a thin film-like growth beyond the enzymic zone forming ear-like lobes.

*Beef agar, 5 days.*

Form: Round or irregularly round.

Size: Surface colonies 2 to 3 mm.; bottom colonies frequently spread over a large part of the plate.

Elevation: Decidedly convex.

Consistency: Soft; old colonies become slightly viscous.

Chromogenesis: White or light grayish white; bottom colonies frequently somewhat fluorescent

Internal structure: Granular

Edge: Entire to undulate

*Potato agar, 5 days.*

Form: Round.

Size: Surface colonies 2 to 3 mm.; bottom colonies may attain a diameter of 10 mm.

Elevation: Distinctly convex; old colonies become somewhat umbilicate.

Consistency: Soft.

Chromogenesis: Glistening grayish white; some colonies show a white nucleus and rim.

Internal structure: Granular; nucleus is more coarsely granular than remainder of colony.

Edge: Entire.

11. *Filter paper broths, 15 days.* The paper is reduced to a disintegrated fibrous mass which retains its pure white color. The destruction takes place at about the same rate with ammonium sulphate, potassium nitrate or peptone as the source of nitrogen. With casein as a source of nitrogen the destruction of the paper is less rapid.

### III. BIOCHEMICAL FEATURES.

12. *Dunham's solution, 10 days* No ammonia produced; no nitrite produced.
13. *Starch nitrate solution, 10 days.* No ammonia produced; nitrite produced.
14. *Peptone nitrite solution, 10 days.* Indol produced.
15. *Carbohydrate broths, 12 days.* No gas produced. Per cent of acidity (Fuller's Scale) with: Dextrose, 1.80; Lactose, .85; Saccharose, 1.30; Maltose, 1.30; Glycerine, .45; Mannite, .00; Starch, 1.35.

*Bacillus desiduus*, n. sp.

SOURCE: Soil from Covina, California, and Riverside, California.

## I. MORPHOLOGY.

1. Vegetative cells: Average dimensions  $1 \times .4 \mu$ .
2. Endospores: None observed.
3. Flagella: 1 to 3 in number; 3 to  $5 \mu$  in length.
4. Staining reactions: Gram negative. Stains readily with the aniline dyes.

## II. CULTURAL CHARACTERISTICS.

5. Agar strokes, 5 days.  
*Beef agar*: Scant, flat, grayish white, filiform growth.  
*Potato agar*: Moderate, dry, cream-colored growth.  
*Peptone starch agar*: Abundant, grayish white growth.
6. *Potato cylinder*: No growth in 30 days.
7. *Gelatin stab*: Moderate grayish white growth at surface and along track of needle, in 5 days; no liquefaction in 30 days.
8. *Beef broth*, 5 days: Lightly clouded.
9. *Litmus milk*: Reddened in 3 days; neither coagulated nor digested in 30 days.
10. Plate cultures.  
*Ammonia cellulose agar*, 15 days.  
Form: Irregularly round.  
Size: Surface colonies are small, rarely becoming more than 1.5 mm. in diameter; bottom colonies frequently attain a diameter of 12 mm.  
Enzymic zone: 2 to 2.5 mm. in 15 days; 3 to 3.5 mm. in 25 days.  
Elevation: Slightly convex.  
Chromogenesis: Colony is gray white with the exception of a small white nucleus and a narrow white rim.  
Structure: Granular.  
Edge: Erode.  
*Peptone cellulose agar*, 15 days.  
Form: Irregularly round.  
Size: Surface colonies 1 to 2 mm.; bottom colonies may attain a diameter of 25 mm.  
Enzymic zone: Surface colonies 1 to 2 mm.; bottom colonies frequently show no enzymic zone until after 20 days.  
Elevation: Slightly convex.  
Chromogenesis: Surface colonies are semi-transparent, yellowish white. After 20 days' growth the surface and imbedded colonies become quite yellowish; bottom colonies remain grayish white.  
Internal structure: Granular.  
Edge: Lobate.  
*Peptone starch agar*, 5 days.  
Form: Surface and bottom colonies are round or irregularly round; imbedded colonies are flaky.  
Size: 1.5 to 2.5 mm.  
Enzymic zone: 1 to 1.5 mm. in 5 days; 2 to 2.5 mm. in 10 days.  
Elevation: Flat.  
Chromogenesis: Grayish white; some colonies show a small white nucleus.

Internal structure: Coarsely granular. The granules are frequently formed into large granular clumps.

Edge: Entire or undulate.

*Beef Agar*, 5 days.

Form: Surface colonies round; bottom colonies spread out into fern-like growths.

Size: Surface colonies 1 to 1.5 mm.; bottom colonies 12 to 15 mm.

Elevation: Slightly convex.

Consistency: Soft; old colonies are somewhat viscous.

Chromogenesis: By reflected light the colonies are grayish white; by transmitted light they appear as glistening semi-transparent drops.

Structure: Granular.

Edge: Entire.

*Potato agar*, 5 days.

Form: Round.

Size: 1 to 1.5 mm.

Elevation: Convex.

Consistency: Very soft; colony can be caused to spread over the medium by shaking the plate.

Chromogenesis: By reflected light the colonies are grayish white. By transmitted light they appear as glistening semi-transparent drops.

Structure: Granular.

Edge: Entire

11. *Filter paper broths*, 15 days. Paper is reduced to a finely divided gray white mass which readily separates into minute fibrous particles on slight agitation. The paper is decomposed rapidly with ammonium sulphate, potassium nitrate, peptone or casein as the source of nitrogen.

### III. BIOCHEMICAL FEATURES.

12. *Dunham's solution*, 10 days. No ammonia formed; nitrite formed.
13. *Starch nitrate solution*, 10 days. No ammonia formed; nitrite formed.
14. *Peptone nitrite solution*, 10 days. Indol produced.
15. *Carbohydrate broths*, 12 days. No gas produced. Per cent of gas (Fuller's Scale) with: Dextrose, .80; Lactose, .10; Saccharose, .00; Maltose, .60; Glycerine, .00; Mannite, .00; Starch, .20.

*Bacillus festinus*, n. sp.

SOURCE: Soil from Banning, California; Fullerton, California; Whittier, California.

### I. MORPHOLOGY.

1. Vegetative cells: Average dimensions  $2 \times .6 \mu$ .
2. Endospores: Form, elliptical; size, average dimensions  $.8 \times .5 \mu$ ; germination, equatorial; rod, swollen.
3. Flagella: 1 to 3 in number; 4 to  $6 \mu$  in length.
4. Staining reactions: Gram negative. Stains readily with the aniline dyes.

### II. CULTURAL CHARACTERISTICS.

5. *Agar strokes*, 5 days.

*Beef agar*: Scant, flat, grayish white, spreading growth.

*Potato agar*: Abundant, grayish white, flat growth, usually spreading over the entire slope.

*Peptone starch agar*: Moderate, grayish white after 5 days, but in cultures from 6 to 10 days old the growth becomes a rich orange. The pigment diffuses through the medium very slowly.

6. *Potato cylinders*: No growth in 30 days.
7. *Gelatin stab*: Scant growth at surface and along track of the needle in 10 days. No liquefaction in 30 days.
8. *Beef broth*: Not clouded in 5 days.
9. *Litmus milk*: Reddened in 3 days; coagulated and digested in 25 days.
10. Plate cultures.

*Ammonia cellulose agar*, 15 days.

Form: Round.

Size: 10 to 12 mm. The colonies continue to grow after 15 days, and when kept in a moist chamber for 30 days the colonies frequently attain a diameter of 25 mm.

Enzymic zone: In young colonies the clearing is all within the colony; after 30 days the enzymic zone is frequently 2 to 3 mm.

Elevation: Saucer-shaped.

Chromogenesis: The central portion of the colony, usually 6 to 10 mm. in diameter, is semi-transparent grayish white. The remainder is vitreous with the exception of a thin white rim.

Internal structure: The central portion of the colony is made up of loosely arranged, coarse granules. The structure of the vitreous zone is indeterminate.

Edge: Entire.

*Peptone cellulose agar*, 15 days.

Form: Round.

Size: 5 to 6 mm. in 15 days; 10 to 12 mm. in 30 days.

Enzymic zone: 2 to 3 mm.

Elevation: Saucer-shaped.

Chromogenesis: Central portion of colony is transparent or semi-transparent grayish white. Outer portion of colony is semi-transparent yellowish white. Colony is usually surrounded by a thin yellowish white rim.

Internal structure: The colony is composed of fine granules loosely arranged.

Edge: Entire.

*Peptone starch agar*, 5 days.

Form: Surface colonies, round; imbedded and bottom colonies irregularly round.

Size: 15 to 25 mm.

Enzymic zone: 2 to 3 mm. in 5 days; 3.5 to 4 mm. in 10 days.

Elevation: Flat or very slightly convex.

Chromogenesis: Central portion of colony is a rich orange, outer portion grayish to yellowish white.

Internal structure: Consists of large granules frequently formed into clumps.

Edge: Entire to undulate.

*Beef Agar*, 5 days.

Form: Round.

Size: Surface colonies 1 mm. or less; bottom colonies 3 to 4 mm.

Elevation: Slightly convex.

Consistency: Butyrous, old colonies become brittle.

Chromogenesis: White nucleus, remainder semi-transparent, glistening, grayish white.

Internal structure: Finely granular with exception of nucleus which is made up of granular clumps.

*Potato agar*, 5 days.

Form: Round.

Size: Surface colonies 2 to 3 mm.; bottom colonies 4 to 5 mm.

Elevation: Convex; old colonies frequently become somewhat umbilicate.

Consistency: Butyrous.

Chromogenesis: Grayish to yellowish white. Sometimes shows brownish rings.

Internal structure: Finely granular

Edge: Entire.

11. *Filter paper broths*, 15 days. Paper is very completely disintegrated into a grayish white felt-like mass, which readily separates into minute fibrous particles on slight agitation. The paper undergoes rapid decomposition when the nutrient solution contains inorganic nitrogen in the form of ammonium sulphate or potassium nitrate, and also when organic nitrogen is added in the form of peptone or casein.

### III. BIOCHEMICAL FEATURES

12. *Dunham's solution*, 10 days. No ammonia produced; no nitrite produced.
13. *Starch nitrate solution*, 10 days. No ammonia produced; nitrite produced.
14. *Peptone nitrite solution*, 10 days. Indol produced.
15. *Carbohydrate broths*, 12 days. No gas produced. Per cent of acidity (Fuller's Scale) with: Dextrose, .50; Lactose, .40; Saccharose, .00; Maltose, .65; Glycerine, .05. Mannite, .00; Starch, .60.

*Bacillus gilvus*, n. sp.

SOURCE: Soil from Azusa, California; Chula Vista, California; Davis, California; Porterville, California; and Riverside, California.

### I. MORPHOLOGY.

1. Vegetative cells: Average dimensions 1.5 x .5  $\mu$ .
2. Endospores: None observed.
3. Flagella: 1 to 4 in number; 4 to 6  $\mu$  in length
4. Staining reactions: Gram negative. Stains readily with the aniline dyes.

### II. CULTURAL CHARACTERISTICS.

5. *Agar strokes*, 5 days.

*Beef agar*: Scant, yellowish white, filiform growth.

*Potato agar*: Abundant, canary yellow, growth spreading over a large part of the slope.

*Peptone starch agar*: Abundant, grayish white, glistening growth which becomes somewhat yellowish after 10 days.

6. *Potato cylinders*: Abundant canary yellow in 5 days.

7. *Gelatin stab*: Moderate yellowish white growth at surface and along track of needle in 10 days; no liquefaction in 30 days.



8. *Beef broth*, 5 days: Slightly clouded.
9. *Litmus milk*: Reddened in 6 days; neither coagulated nor digested in 30 days.
10. Plate cultures.
  - Ammonia cellulose agar*, 15 days.
    - Form: Round to irregularly round.
    - Size: 2 to 3 mm.
    - Enzymic zone: Entire colony semi-transparent; enzymic zone not more than 1 mm.
    - Elevation: Flat or slightly depressed.
    - Chromogenesis: Semi-transparent, grayish white, usually showing a small white nucleus.
    - Internal structure: Granular.
    - Edge: Entire to undulate.
  - Peptone cellulose agar*, 15 days.
    - Form: Round.
    - Size: 2 to 4 mm.
    - Enzymic zone: 1.5 to 2 mm in 15 days; 3 to 4 mm. in 25 days.
    - Elevation: Slightly concave.
    - Chromogenesis: Grayish white, frequently showing a small white nucleus, usually forms a thin grayish white semi-transparent rim beyond the enzymic zone.
    - Internal structure: Granular.
    - Edge: Entire.
  - Peptone starch agar*, 5 days.
    - Form: Round to irregularly round.
    - Size: 2 to 3 mm.
    - Enzymic zone: 1 to 1.5 mm.
    - Elevation: Slightly convex.
    - Consistency: Soft, becoming brittle after 10 days.
    - Chromogenesis: Grayish to yellowish white. After 10 days the colonies become quite yellowish.
    - Internal structure: Granular.
    - Edge: Entire to undulate.
  - Beef Agar*, 5 days.
    - Form: Round.
    - Size: Surface colonies 1 to 2 mm.; bottom colonies 3 to 3.5 mm.
    - Elevation: Convex.
    - Consistency: Soft.
    - Chromogenesis: After 3 days the colonies are grayish to yellowish white; the yellow color increases with the age of the colony and after 10 days they are distinctly yellow.
    - Internal structure: Coarsely granular. The granules are frequently formed into clumps.
    - Edge: Entire.
  - Potato agar*, 5 days.
    - Form: Round.
    - Size: 2 to 3 mm.
    - Elevation: Convex.
    - Consistency: Butyrous.
    - Chromogenesis: Canary yellow; some colonies show brownish rings.
    - Internal structure: Granular.
    - Edge: Entire.

11. *Filter paper broths*, 15 days. The paper is reduced to a thin white filmy mass which breaks up into minute particles on slight agitation. The decomposition of the paper proceeds rapidly with ammonium sulphate, potassium nitrate, peptone or casein as the source of nitrogen.

### III. BIOCHEMICAL FEATURES.

12. *Dunham's solution*, 10 days. Ammonia formed; nitrite formed.
13. *Starch nitrate solution*, 10 days. No ammonia formed; nitrite formed.
14. *Peptone nitrite solution*, 10 days. Indol formed.
15. *Carbohydrate broths*, 12 days. No gas produced. Per cent of acidity (Fuller's Scale) with: Dextrose, 1.20; Lactose, .75; Saccharose, .80; Maltose, 1.00; Glycerine, .40; Mannite, .00; Starch, 1.00.

*Bacillus imminutus*, n. sp.

**SOURCE:** Soil from Highland, California; Berkeley, California; Corona, California; Redlands, California; Whittier, California; Santa Paula, California; Pasadena, California; Azusa, California; Fullerton, California; Porterville, California.

### I. MORPHOLOGY.

1. Vegetative cells: Average dimensions  $1.5 \times 2 \mu$ . The vegetative cells pass quickly into involution forms which frequently attain a length of from 6 to  $8 \mu$  without increasing in thickness. The involution forms are commonly curved cells, frequently more or less fusiform.
2. Endospores: Form, round; average size,  $.5 \mu$ ; germination, polar. Rod is swollen during germination, giving the cell a drumstick appearance. On cellulose agar the spores appear in from 4 to 6 days.
3. Flagella: 1 to 5 in number;  $3$  to  $5 \mu$  in length.
4. Staining reactions: Gram negative. Stains readily with the aniline dyes.

### II. CULTURAL CHARACTERISTICS.

5. Agar strokes, 5 days.  
*Beef agar*: No growth.  
*Potato agar*: No growth.  
*Peptone starch agar*: No growth.
6. *Potato cylinders*: No growth in 30 days.
7. *Gelatine stab*: No growth in 30 days.
8. *Beef broth*, 5 days: No growth.
9. *Litmus milk*: No growth in 30 days.
10. Plate cultures.  
*Ammonia cellulose agar*, 15 days.  
Form: Round.  
Size: The size of the colonies is quite variable; after 15 days the diameter is usually between 12 and 15 mm. The colony continues to increase in size as long as the medium remains moist, and where plates can be kept free from molds a single colony may eventually cover the entire plate. The round form of the colony is maintained as long as the growth is unobstructed.

**Enzymic zone:** The entire colony is transparent. The enzyme does not clear the cellulose beyond the development of the colony.

**Elevation:** Young colonies are saucer-shaped. As the colony spreads the depression is less apparent.

**Chromogenesis:** Vitreous. Some colonies show a very narrow white rim. Old colonies frequently become a light transparent yellow.

**Internal structure:** Granular.

**Edge:** Entire.

*Peptone cellulose agar*, 15 days.

**Form:** Round.

**Size:** 10 to 12 mm. in 15 days; colonies continue to grow as long as the medium is kept moist. When the plate contains only a very few colonies the diameter may be 50 mm. or more in 30 days.

**Enzymic zone:** The entire colony is transparent with the exception of a very narrow white rim. The enzyme does not spread beyond the development of the colony.

**Elevation:** The young colonies are distinctly concave. As the colony becomes older the depression is less apparent.

**Chromogenesis:** Vitreous. Some colonies show a very narrow white rim. Old colonies frequently become a light transparent yellow.

**Internal structure:** Indeterminate with the exception of the narrow white rim which is granular.

**Edge:** Entire.

*Peptone starch agar:* No colonies produced in 10 days.

*Beef agar:* No colonies produced in 10 days.

*Potato agar:* No colonies produced in 10 days.

11. *Füiter paper broths*, 15 days. The paper is reduced to a very thin yellowish filmy mass, which disintegrates on very slight agitation. The paper is destroyed at about the same rate with ammonium sulphate, potassium nitrate or peptone as the source of nitrogen. A slower destruction of the paper occurs when nitrogen is supplied in the form of casein.

### III. BIOCHEMICAL FEATURES.

12. *Dunham's solution*, 10 days. No ammonia produced; no nitrite produced.
13. *Starch nitrate solution*, 10 days. No ammonia produced; no nitrite produced.
14. *Peptone nitrite solution*, 10 days. No indol produced.
15. *Carbohydrate broths*, 12 days. No gas produced. Per cent of acidity (Fuller's Scale) with: Dextrose, 0.00; Lactose, 0.00; Saccharose, 0.00; Maltose, 0.00; Glycerine, 0.00; Mannite, 0.00; Starch, 0.00.

### *Bacillus iugis*, n. sp.

**SOURCE:** Soil from Lordsburg, California; Redlands, California; and San Fernando, California.

#### I. MORPHOLOGY.

1. Vegetative cells: Average dimensions  $1.4 \times .4 \mu$ .
2. Endospores: None observed.

3. **Flagella:** 1 to 3 in number; 3 to 4  $\mu$  in length.
4. **Staining reactions:** Gram negative. Stains readily with the aniline dyes.

## II. CULTURAL CHARACTERISTICS.

5. **Agar strokes, 5 days.**

*Beef agar:* Scant, grayish white, filiform growth.

*Potato agar:* Abundant, glistening, grayish white, filiform growth.

*Peptone starch agar:* Moderate, grayish white, filiform growth.

6. *Potato cylinders, 30 days:* Scant, glistening, colorless growth when very heavily inoculated. Light inoculation produces no growth.
7. *Gelatin stab:* Moderate growth at surface and along track of needle in 5 days; napiform liquefaction in 30 days.
8. *Beef broth, 5 days:* Heavily clouded.
9. *Litmus milk:* Reddened in 5 days; neither coagulated nor digested in 30 days.
10. **Plate cultures.**

*Ammonia cellulose agar, 15 days.*

Form: Round.

Size: 1.5 to 2.5 mm. in 15 days; 3 to 3.5 mm. in 25 days.

Enzymic zone: Clearing sometimes all within colony after 15 days. After 20 days all colonies show an enzymic zone of 1 mm. or more.

Elevation: Flat.

Chromogenesis: Semi-transparent, light grayish white; sometimes contoured by light whitish lines

Internal structure: Granular.

Edge: Entire.

*Peptone cellulose agar, 15 days.*

Form: Irregularly round.

Size: 5 to 8 mm. in 15 days; no increase in size after 15 days.

Enzymic zone: 2 to 3 mm. The zone continues to increase in width up to 30 days, in which time it is frequently 5 mm.

Elevation: Slightly convex.

Chromogenesis: Central portion of colony is white; the outer portion gray-white; sometimes forms a white nucleus and rim.

Internal structure: Central portion of colony coarsely granular, outer portion finely granular.

Edge: Undulate to lobate.

*Peptone starch agar, 5 days.*

Form: Irregularly round.

Size: 1.5 to 2 mm. in 15 days; 2.5 mm. in 25 days.

Enzymic zone: 1 mm. or less.

Elevation: Capitate. (The colonies on starch agar are raised in a characteristic jelly-like mass.)

Consistency: Gelatinous.

Chromogenesis: Grayish white.

Internal structure: Fine granules loosely arranged.

Edge: Lanceolate.

*Beef agar, 5 days.*

Form: Surface colonies round; imbedded colonies, lenticular.

Size: 1.5 to 2 mm.

Elevation: Convex.

Consistency: Soft; old colonies become somewhat gelatinous.

Chromogenesis: Small white nucleus, remainder semi-transparent grayish white.

Internal structure: Granular.

Edge: Entire.

*Potato agar*, 5 days.

Form: Surface colonies, round; bottom colonies, irregularly round.

Size: Surface colonies 1 to 1.5 mm.; bottom colonies 2.5 to 4 mm.

Elevation: Convex.

Consistency: Soft.

Chromogenesis: Grayish white, with a pearl-like luster.

Internal structure: Granular.

Edge: Entire.

11. *Filter paper broths*, 15 days. The paper retains something of its original structure; but shows many ragged holes where the fibers have been dissolved away. Very slight agitation is sufficient to disintegrate the paper mass completely. The decomposition takes place at about the same rate with ammonium sulphate or peptone as the source of nitrogen. The decomposition was much slower when casein or potassium nitrate was used.

### III. BIOCHEMICAL FEATURES.

12. *Dunham's solution*, 10 days. Ammonia produced; nitrite produced.
13. *Starch nitrate solution*, 10 days. No ammonia produced; nitrite produced.
14. *Peptone nitrite solution*, 10 days. No indol produced.
15. *Carbohydrate broths*, 12 days. No gas produced. Per cent of acidity (Fuller's Scale) with: Dextrose, .80; Lactose, 1.10; Saccharose, 1.60; Maltose, 1.55; Glycerine, .45; Mannite, .20; Starch, 1.50.

### *Bacterium castigatum*, n. sp.

SOURCE: Soil from Banning, California; Glendora, California; and Wineville, California.

#### I. MORPHOLOGY.

1. Vegetative cells: Average dimensions  $1.2 \times .4 \mu$ .
2. Endospores: None observed.
3. Staining reactions: Gram negative. Stains readily with the aniline dyes.

#### II. CULTURAL CHARACTERISTICS.

4. *Agar strokes*, 5 days.  
*Beef agar*: Abundant, moist, glistening, grayish white growth.  
*Potato agar*: Abundant, glistening, grayish white; becomes yellowish white after 10 days.  
*Peptone starch agar*: Abundant, raised, somewhat rugose.
5. *Potato cylinders*, 30 days: No growth.
6. *Gelatin stab*: Moderate growth at surface and along track of needle in 6 days; no liquefaction after 30 days.
7. *Beef broth*, 5 days: Lightly clouded.
8. *Litmus milk*: Reddened in 3 days; neither coagulated nor digested in 30 days.

## 9. Plate cultures.

*Ammonia cellulose agar*, 15 days.

Form: Irregularly round.

Size: 1 to 1.5 mm.

Enzymic zone: 1 to 1.5 mm.; in 30 days the enzymic zone may attain a diameter of 2.5 mm.

Elevation: Slightly convex.

Chromogenesis: Opaque white or light grayish white.

Internal structure: Granular.

Edge: Undulate.

*Peptone cellulose agar*, 15 days.

Form: Irregularly round.

Size: 1 to 1.5 mm.

Enzymic zone: .5 to 1 mm.; in 30 days the enzymic zone may reach a diameter of 2 mm.

Elevation: Slightly convex.

Chromogenesis: White nucleus and rim, remainder of colony grayish white.

Internal structure: Nucleus and rim made up of coarse compact granules, remainder of colony finely granular.

Edge: Undulate.

*Peptone starch agar*, 5 days.

Form: Irregularly round.

Size: 7 to 10 mm.

Enzymic zone: 1 to 1.5 mm. in 5 days; 2.5 to 3 mm. in 10 days.

Elevation: Flat or slightly convex.

Consistency: Firm.

Chromogenesis: Grayish white cottony-like colony in 5 days; in 10 days colonies become distinctly grayish.

Internal structure: Coarsely granular.

Edge: Lancelate.

*Beef agar*, 5 days

Form: Round.

Size: Surface colonies 1 to 1.5 mm.; bottom colonies 2 to 3 mm.

Elevation: Slightly convex.

Consistency: After 5 days colonies are soft; after 10 days, brittle.

Chromogenesis: Very small white nucleus, remainder of colony grayish white. Surface colonies exhibit a pearl-like luster.

Internal structure: Granular.

Edge: Entire.

*Potato agar*, 5 days.

Form: Surface colonies round; bottom colonies irregularly round.

Size: Surface colonies 1 to 2 mm.; bottom colonies 2 to 3 mm.

Elevation: Convex.

Consistency: After 5 days colonies are soft; after 10 days, butyrous.

Chromogenesis: Light grayish white, semi-transparent colonies with a pearl-like luster.

Internal structure: Made up of fine granules, loosely arranged.

Edge: Entire.

10. *Filter paper broths*, 15 days. Paper very completely disintegrated and reduced to a pulp-like mass which settles to the bottom of the flask. The paper is vigorously attacked in solutions containing ammonium sulphate, potassium nitrate, or peptone as the source of nitrogen. Casein appeared to be less favorable for the rapid development of this organism.

### III. BIOCHEMICAL FEATURES.

11. *Dunham's solution*, 10 days. No ammonia formed; nitrite formed.
12. *Starch nitrate broth*, 10 days. No ammonia formed; no nitrite formed.
13. *Peptone nitrite broth*, 10 days. No indol produced.
14. *Carbohydrate broths*. No gas produced. Per cent of acidity (Fuller's Scale) with: Dextrose, 1.50; Lactose, 1.10; Saccharose, 1.00; Maltose, 1.45; Glycerine, .55; Mannite, .00; Starch, 1.40.

### *Bacterium idoneum*, n. sp.

SOURCE: Soil from Mentone, California; and Whittier, California.

#### I. MORPHOLOGY.

1. Vegetative cells: Average dimensions 1.5 to .5  $\mu$ .
2. Endospores: None observed.
3. Staining reactions: Gram negative. Stains readily with the aniline dyes.

#### II. CULTURAL CHARACTERISTICS.

4. Agar strokes, 5 days.  
*Beef agar*: Scant, yellowish white, glistening, filiform growth; in 10 days growth becomes distinctly yellowish.  
*Potato agar*: Abundant, moist, glistening, faint yellowish to glistening white; becomes distinctly yellowish in 10 days.  
*Peptone starch agar*: Moderate, flat, white, filiform growth; becomes faintly yellowish in 10 days.
5. *Potato cylinders*: Abundant, moist, glistening, grayish white growth in 15 days.
6. *Gelatin stab*: Moderate, yellowish growth at surface and along track of needle in 10 days. Slight napiform liquefaction after 30 days.
7. *Beef broth*, 5 days: Turbid.
8. *Litmus milk*: Reddened in 3 days; neither coagulated nor digested in 30 days.
9. Plate cultures.  
*Ammonia cellulose agar*, 15 days.  
 Form: Irregularly round.  
 Size: 1 to 1.5 mm.  
 Enzymic zone: 1 mm. or less after 15 days; after 30 days the enzymic zone has attained a diameter of 2 to 3 mm.  
 Elevation: Flat.  
 Chromogenesis: Opaque white or light grayish white.  
 Internal structure: The colony is made up of rather coarse granules compactly arranged.  
 Edge: Lobate.  
*Peptone cellulose agar*, 15 days.  
 Form: Irregularly round.  
 Size: 1 to 1.5 mm.; maximum development is reached in 15 days.  
 Enzymic zone: .5 to 1.0 mm. in 15 days; 1.5 to 2 mm. after 30 days.  
 Elevation: Flat.

Chromogenesis: Opaque white to light grayish white.

Internal structure: Coarse granules, compactly arranged.

Edge: Lobate.

*Peptone starch agar*, 5 days.

Form: Irregularly round.

Size: 1 to 2 mm.

Enzymic zone: 1 to 1.5 mm. in 5 days; 2 to 2.5 mm. in 10 days.

Elevation: Convex; frequently somewhat pulvinate.

Consistency: After 5 days the colonies are soft; older colonies become somewhat viscous.

Internal structure: Granular; granules frequently arranged in clumps.

Edge: Lobate.

*Beef agar*, 5 days.

Form: Round.

Size: Surface colonies 1 to 1.5 mm.; bottom colonies 2 to 3 mm.

Elevation: Convex.

Consistency: Soft; becomes brittle after 10 days.

Chromogenesis: Grayish white pearl-like luster. By transmitted light the colonies appear as semi-transparent glistening drops.

Internal structure: Granular.

Edge: Entire.

*Potato agar*, 5 days.

Form: Surface colonies, round; imbedded and bottom colonies, irregularly round.

Size: 2 to 3 mm.

Elevation: Pulvinate.

Consistency: After 5 days colonies are soft; after 10 days, butyrous or brittle.

Chromogenesis: Reflected light, yellowish to grayish white; transmitted light, semi-transparent glistening drops.

Internal structure: Coarsely granular.

Edge: Entire.

10. *Filter paper broths*, 15 days Paper is reduced to a thin limp sheet which falls apart on slight agitation. Solutions containing ammonium sulphate, potassium nitrate and peptone as the source of nitrogen show a rapid decomposition of the paper. Solutions containing casein showed only a slight decomposition of the paper even after 30 days' incubation.

### III. BIOCHEMICAL FEATURES.

11. *Dunham's solution*, 10 days. No ammonia formed; nitrite formed.
12. *Starch nitrate broth*, 10 days. No ammonia produced; nitrite produced.
13. *Peptone nitrite solution*, 10 days. No indol produced.
14. *Carbohydrate broths*, 12 days. No gas produced. Per cent of acidity (Fuller's Scale) with: Dextrose, 1.60; Lactose, 1.20; Maltose, 1.40; Mannite, .00; Saccharose, 1.30; Glycerine, .70; Starch, 1.40.

*Bacterium lucrosum*, n. sp.

SOURCE: Soil from Redlands, California; and Upland, California.

#### I. MORPHOLOGY.

1. Vegetative cells: Average dimensions 1.3. x .4  $\mu$ .
2. Endospores: None observed.



3. Staining reactions: Gram negative. Stains readily with the aniline dyes.

## II. CULTURAL CHARACTERISTICS.

4. Agar strokes, 5 days.

*Beef agar*: Moderate, flat, grayish white; old cultures become somewhat iridescent.

*Potato agar*: Moderate, dirty yellowish white in 5 days; becomes more yellowish with age.

*Peptone starch agar*: Abundant, moist, grayish white in 5 days; becomes faintly yellowish in 10 days.

5. *Potato cylinder*, 30 days: No growth.
6. *Gelatin stab*: No growth after 30 days.
7. *Beef broth*, 5 days: Heavily clouded.
8. *Litmus milk*: No change in milk in 30 days.
9. Plate cultures.

*Ammonia cellulose agar*, 15 days.

Form: Irregularly round.

Size: 15 to 20 mm.; in 30 days the colonies frequently reach a diameter of 25 to 30 mm.

Enzymic zone: When there are only a few colonies on the plate, permitting rapid spreading, the clearing is all within the colony until after 30 days, when an enzymic zone usually develops. On crowded plates the colonies always show an enzymic zone of 1 mm. or more.

Elevation: Slightly concave.

Chromogenesis: Central portion of colony, usually 6 to 19 mm. in diameter, is grayish white; outer portion of colony is vitreous. The vitreous zone is usually surrounded by a thin white rim.

Internal structure: Colony is made up of medium-sized, loosely arranged granules.

Edge: Undulate.

*Peptone cellulose agar*, 15 days.

Form: Round.

Size: 2 to 3 mm. in 15 days; 3 to 4 mm. in 25 days.

Enzymic zone: 1 to 1.5 mm. in 15 days; 2 to 3 mm. in 25 days.

Elevation: Flat.

Chromogenesis: Nucleus and rim are white, remainder of colony semi-transparent grayish white.

Internal structure: Granular.

Edge: Entire.

*Peptone starch agar*, 5 days.

Form: Irregularly round.

Size: 3 to 4 mm.

Enzymic zone: .5 to 1 mm. in 5 days; 2 to 3 mm. in 10 days.

Elevation: Convex.

Consistency: Soft; after 10 days colonies become brittle.

Chromogenesis: Central portion of colony semi-transparent, glistening white; outer portion vitreous.

Internal structure: Granular.

Edge: Undulate.

**Beef agar, 5 days.**

Form: Surface colonies, round; imbedded colonies, lenticular; bottom colonies, irregularly round.

Size: 1 to 1.5 mm.

Elevation: Slightly convex.

Consistency: Soft; in 10 days growth becomes somewhat gelatinous.

Chromogenesis: Very small white nucleus; remainder of colony semi-transparent grayish white.

Internal structure: Granular.

Edge: Entire.

**Potato agar, 5 days.**

Form: Surface colonies, round; bottom and imbedded colonies, irregularly round.

Size: 1.5 to 2 mm.

Elevation: Convex

Consistency: Butyrous after 5 days; somewhat gelatinous after 10 days.

Chromogenesis: Yellowish to grayish white; after 10 days colonies become quite yellowish.

Internal structure. Coarsely granular. Some colonies are grumose

Edge: Entire

10. *Filter paper broths*, 15 days. Paper is reduced to pulpy grayish white mass consisting of very short fibers which separate on slight agitation. The paper is decomposed rapidly in the ammonium sulphate and peptone broths, but more slowly in the broths containing casein or potassium nitrate.

**III. BIOCHEMICAL CHARACTERISTICS.**

11. *Dunham's solution*, 10 days. No ammonia produced; nitrite produced.
12. *Starch nitrate solution*, 10 days. No ammonia produced; no nitrite produced.
13. *Peptone nitrite solution*, 10 days. No indol produced.
14. *Carbohydrate broths*. No gas produced. Per cent of acidity (Fuller's Scale) with: Dextrose, .20; Lactose, .10; Saccharose, .00; Maltose, .15; Glycerine, .00; Mannite, .05; Starch, .15.

*Bacterium paludosum*, n. sp.

SOURCE: Soil from Berkeley, California; Whittier, California.

**I. MORPHOLOGY.**

1. Vegetative cells: Average dimensions  $1.5 \times .4 \mu$ .
2. Endospores: Form, elliptical; size,  $1.2 \times .6 \mu$ ; germination, equatorial; rod, swollen. Abundantly produced on potato agar cultures 3 or 4 days old.
3. Staining reactions: Gram negative. Stains readily with the aniline dyes.

**II. CULTURAL CHARACTERISTICS**

4. Agar strokes, 5 days.

*Beef agar*: Moderate, flat, grayish white growth.

*Potato agar*: Abundant, moist, glistening, grayish white growth.

*Peptone starch agar*: Moderate, flat, grayish white growth.

5. *Potato cylinder*: Very scant, glistening, colorless growth sometimes occurs on cylinders held in a moist chamber for 30 days, but ordinarily no growth is secured.
6. *Gelatin stab*: Moderate growth at surface and along track of needle in 6 days. After 30 days napiform liquefaction is observed.
7. *Beef broth*, 5 days: Lightly clouded.
8. *Litmus milk*: Reddened in 5 days; neither coagulated nor digested in 30 days.
9. Plate cultures.

*Ammonia cellulose agar*, 15 days.

Form: Surface colonies, round or irregularly round; bottom colonies frequently develop into fern-like growths.

Size: Surface colonies, 2 to 3 mm.; bottom colonies frequently attain a diameter of 8 to 10 mm.

Enzymic zone: 1 to 3 mm. in 15 days; 3 to 3.5 mm. in 25 days.

Elevation: Slightly convex.

Chromogenesis: Surface colonies show a small white nucleus; remainder of colony, gray-white; bottom colonies are fluorescent.

Internal structure: Coarsely granular.

Edge: Entire to undulate.

*Peptone cellulose agar*, 15 days.

Form: Irregularly round.

Size: Surface colonies 1.5 to 2.5 mm.; bottom colonies 3 to 5 mm.

Enzymic zone: 1.5 to 2 mm. in 15 days; 2.5 to 3 mm. in 30 days.

Elevation: Convex.

Chromogenesis: White to grayish white. Colonies sometimes show a white nucleus and rim.

Internal structure: Coarsely granular.

Edge: Lacerate.

*Peptone starch agar*, 5 days.

Form: Irregular; imbedded colonies frequently throw out spine-like growths.

Size: 1.5 to 2 mm.

Enzymic zone: 1.5 to 2 mm. in 5 days; 2.5 to 3.5 mm. in 10 days.

Elevation: Flat.

Chromogenesis: White to light grayish white in 5 days; in 10 days the colonies become dark gray.

Internal structure: Densely granular.

Edge: Lacerate.

*Beef agar*, 5 days.

Form: Surface colonies round; bottom colonies spread out into irregular growths.

Size: Surface colonies 1.5 to 2 mm.; bottom colonies 10 to 12 mm.

Consistency: Very soft in 5 days; brittle in 10 days.

Chromogenesis: Semi-transparent, glistening, gray-white; frequently form a small white nucleus, and many colonies are more or less concentric in structure. At an angle of 45° the colonies are fluorescent.

Internal structure: Granular.

Edge: Entire to undulate.

*Potato agar*, 5 days.

Form: Round.

Size: 2 to 3 mm.; bottom colonies are no larger than the surface colonies.

Elevation: Decidedly convex; in 10 days colonies frequently become somewhat umbilicate.

Consistency: Butyrous.

Chromogenesis: Semi-transparent, glistening, light grayish white to almost vitreous. Many colonies exhibit a pearl-like luster.

Internal structure: Finely granular.

Edge: Entire.

10. *Filter paper broths*, 15 days. Paper is reduced to a white pulp-like mass made up of very short disintegrated fibers which become distributed through the solution on slight agitation. The paper is decomposed very rapidly with ammonium sulphate, potassium nitrate, and peptone as the source of nitrogen. The decomposition takes place more slowly when casein is added as the source of nitrogen.

### III. BIOCHEMICAL FEATURES.

11. *Dunham's solution*, 10 days. No ammonia produced; nitrite produced.  
12. *Starch nitrate solution*, 10 days. No ammonia produced; no nitrite produced.  
13. *Peptone nitrite solution*, 10 days. Indol produced.  
14. *Carbohydrate broths*, 12 days. No gas produced. Per cent of acidity (Fuller's Scale) with: Dextrose, 1.10; Lactose, .80; Saccharose, 1.00; Maltose, 1.20; Glycerine, .40; Mannite, .05; Starch, 1.20.

### *Pseudomonas arguta*, n. sp.

SOURCE: Soil from Azusa, California; and Whittier, California.

#### I. MORPHOLOGY.

1. Vegetative cells: Average dimensions  $.8 \times .3 \mu$ .  
2. Endospores: None observed.  
3. Flagella: 1 to 2 in number; 6 to  $8 \mu$  in length.  
4. Staining reactions: Gram negative. Stains readily with the aniline dyes.

#### II. CULTURAL CHARACTERISTICS

5. Agar strokes, 5 days.  
*Beef agar*: Scant, grayish white, filiform growth.  
*Potato agar*: Moderate, yellowish, glistening white.  
*Peptone starch agar*: Scant, white to grayish white.  
6. *Potato cylinders*, 30 days. No growth.  
7. *Gelatin stab*: Moderate yellowish growth at surface and along the track of needle in 10 days; no liquefaction in 30 days.  
8. *Beef broth*, 5 days: Clouded.  
9. *Litmus milk*: Reddened in 4 days; neither coagulated nor digested in 30 days.  
10. Plate cultures.  
*Ammonia cellulose agar*, 15 days.  
Form: Round.  
Size: Surface colonies, 1 to 2 mm.; bottom colonies, 3 to 4 mm.  
Enzymic zone: 1 mm. or less in 15 days; in 30 days the zone frequently becomes 2 or 3 mm.

Elevation: Slightly convex.

Chromogenesis: White nucleus and rim; remainder semi-transparent grayish white.

Structure: Grumose.

Edge: Entire.

*Peptone cellulose agar*, 15 days.

Form: Round.

Size: 8 to 12 mm. in 15 days; in 30 days the colonies frequently attain a diameter of 20 mm.

Enzymic zone: 1 to 2 mm.

Elevation: Slightly convex.

Chromogenesis: Central portion, usually from 5 to 7 mm. in diameter, is yellowish white. The central portion of the colony is surrounded by a vitreous zone, which in turn is surrounded by a light grayish white rim.

Internal structure: Granular.

Edge: Erode.

*Peptone starch agar*, 5 days.

Form: Irregularly round.

Size: 5 to 8 mm.

Enzymic zone: 1 to 2 mm. in 5 days; 3 to 4 mm. in 10 days.

Elevation: Flat.

Consistency: Soft in 5 days; older colonies become firm.

Chromogenesis: Central portion, usually 2 to 3 mm. in diameter, opaque white. The opaque portion of the colony is surrounded by a vitreous zone, which in turn is surrounded by a thin semi-transparent grayish white rim.

Internal structure: Granular.

Edge: Undulate.

*Beef agar*, 5 days.

Form: Round.

Size: Surface colonies, 1 to 1.5 mm.; bottom colonies, 2 to 3 mm.

Elevation: Slightly convex.

Consistency: Soft to butyrous.

Chromogenesis: Reflected light, grayish white; transmitted light, the colonies appear as semi-transparent glistening drops.

Internal structure: Granular.

Edge: Entire.

*Potato agar*, 5 days.

Form: Round.

Size: 1 to 2 mm.

Elevation: Convex.

Consistency: Very soft.

Chromogenesis: Grayish white, frequently develops brownish rings.

Internal structure: Granular.

Edge: Entire.

11. *Filter paper broths*, 15 days. Paper is reduced to a loose flocculent mass which disintegrates very readily on slight agitation. Paper is decomposed rapidly when the broths contain ammonium sulphate, potassium nitrate, peptone, or casein as the source of nitrogen.

## III. BIOCHEMICAL FEATURES.

12. *Dunham's solution*, 10 days. No ammonia formed; nitrite formed.
13. *Starch nitrate solution*, 10 days. No ammonia formed; no nitrite formed.
14. *Peptone nitrite solution*, 10 days. No indol formed.
15. *Carbohydrate broths*, 12 days. No gas produced. Per cent of acidity (Fuller's Scale) with: Dextrose, .30; Lactose, .10; Saccharose, .00; Maltose, .20; Glycerine, .00; Mannite, .00; Starch, .30.

*Pseudomonas minuscula*, n. sp.

SOURCE: Soil from Bonita, California; Lordsburg, California; and Sanger, California.

## I. MORPHOLOGY.

1. Vegetative cells: Average dimensions,  $.9 \times .5 \mu$ .
2. Endospores: None observed.
3. Flagella: 1, rarely 2 in number; 3 to 4  $\mu$  in length.
4. Staining reactions: Gram positive. Stains readily with the aniline dyes.

## II. CULTURAL CHARACTERISTICS.

5. Agar strokes, 5 days.  
*Beef agar*: Moderate, flat, grayish white, filiform growth.  
*Potato agar*: Abundant, moist, glistening, grayish to yellowish white.  
*Peptone starch agar*: Moderate, grayish white, filiform growth.
6. *Potato cylinders*: No apparent growth after 30 days, but potato is bleached along the track of the inoculating needle.
7. *Gelatin stab*: Moderate growth at surface and along track of needle in 6 days; slight napiform liquefaction after 30 days.
8. *Beef broth*, 5 days: Turbid.
9. *Litmus milk*: Reddened in 6 days; neither coagulated nor digested in 30 days.
10. Plate cultures.  
*Ammonia cellulose agar*, 15 days.  
Form: Round or irregularly round.  
Size: Surface colonies 1 to 2 mm.; bottom colonies may attain a diameter of 6 to 10 mm.  
Enzymic zone: 1.5 to 2 mm.  
Elevation: Slightly depressed.  
Consistency: Soft.  
Chromogenesis: Nucleus and rim are white, remainder of colony grayish white.  
Internal structure: Granular.  
Edge: Undulate.  
*Peptone cellulose agar*, 15 days.  
Form: Round or irregularly round.  
Size: 1 to 2 mm.; bottom colonies frequently attain a diameter of 10 mm.  
Enzymic zone: 1 to 1.50 mm.  
Elevation: Slightly convex.  
Consistency: Soft.  
Chromogenesis: White to grayish white.  
Internal structure: Granular.  
Edge: Erode.

*Peptone starch agar*, 5 days.

Form: Irregular, round.

Size: 1 to 1.5 mm.

Enzymic zone: 2 to 3 mm.

Elevation: Slightly convex.

Consistency: Firm.

Chromogenesis: White to light grayish white.

Internal structure: Granular.

Edge: Lacerate.

*Beef agar*, 5 days.

Form: Round.

Size: 1 mm. or less.

Elevation: Slightly convex.

Consistency: Butyrous; old colonies become brittle.

Chromogenesis: By reflected light colonies are gray white; by transmitted light they appear as light, semi-transparent smoky drops.

Internal structure: Finely granular.

Edge: Entire.

*Potato agar*, 5 days.

Form: Round.

Size: 1 to 2 mm.

Elevation: Convex.

Consistency: Soft.

Chromogenesis: Gray-white, sometimes showing concentric structure.

Internal structure: Granular.

Edge: Entire.

11. *Filter paper broths*, 15 days. Paper reduced to a felt-like grayish white mass which breaks up into small particles on very slight agitation. Paper destroyed more rapidly in solutions containing ammonium sulphate, peptone, or potassium nitrate than in solutions containing casein.

## III. BIOCHEMICAL FEATURES.

12. *Dunham's solution*, 10 days. No ammonia produced; nitrite produced.
13. *Starch nitrate broth*, 10 days. No ammonia produced; nitrite produced.
14. *Peptone nitrite solution*, 10 days. Indol produced.
15. *Carbohydrate broths*. No gas produced. Per cent of acidity (Fuller's Scale) with: Dextrose, 1.20; Lactose, 1.10; Saccharose, 1.00; Maltose, 1.10; Glycerine, .00; Mannite, .00; Starch, .90.

*Pseudomonas mira*, n. sp.

SOURCE: Soil from Corona, California; Glendora, California; Monrovia, California.

## I. MORPHOLOGY.

1. Vegetative cells: Average dimensions,  $1.6 \times .4 \mu$ .
2. Endospores: None observed.
3. Flagella: 1 in number; 4 to 6  $\mu$  in length.
4. Staining reactions: Gram negative. Stains readily with the aniline dyes.

## II. CULTURAL CHARACTERISTICS.

5. Agar strokes, 5 days.

*Beef agar*: Moderate, flat, grayish white, somewhat iridescent.

*Potato agar*: Abundant, grayish white; becomes grayish brown in 10 days.

*Peptone starch agar*: Abundant, moist, grayish white; in 10 days the growth at the bottom of the slope becomes flesh colored.

6. *Potato cylinder*: Moderate, grayish white, leathery growth in 15 days.
7. *Gelatin stab*: Good growth at surface and along track of needle in 6 days; no liquefaction in 30 days.
8. *Beef broth*, 5 days: Heavily clouded.
9. *Litmus milk*: Blued in 10 days; neither coagulated nor digested in 30 days.
10. Plate cultures.

*Ammonia cellulose agar*, 15 days.

Form: Round or irregularly round.

Size: 1 to 1.5 mm.

Enzymic zone: 1 to 2 mm. in 15 days; 3 to 4 mm. in 30 days.

Elevation: Slightly convex.

Chromogenesis: Surface colonies opaque white; bottom colonies semi-transparent grayish white.

Internal structure: Granular.

Edge: Erode.

*Peptone cellulose agar*, 15 days.

Form: Round to irregularly round.

Size: Surface colonies, 1 to 2 mm.; bottom colonies 6 to 8 mm.

Enzymic zone: 1 mm. or less in 15 days; 2 to 3 mm. in 30 days.

Elevation: Slightly convex.

Chromogenesis: Surface colonies have a small white nucleus, remainder of colonies grayish white.

Internal structure: Granular.

Edge: Entire to undulate.

*Peptone starch agar*, 5 days.

Form: Irregularly round.

Size: 2 to 3 mm.

Enzymic zone: 1.5 to 2 mm.

Elevation: Slightly convex.

Consistency: Firm.

Chromogenesis: White to light grayish white; sometimes shows a small white nucleus.

Internal structure: Granular.

Edge: Lacerate.

*Beef agar*, 5 days.

Form: Surface colonies are round; bottom colonies spread profusely.

Size: 2 to 3 mm.

Elevation: Convex.

Consistency: Soft to butyrous.

Chromogenesis: Small white nucleus, remainder gray-white.

Internal structure: Granular.

Edge: Lacerate.

*Potato agar*, 5 days.

Form: Round.

Size: 2 to 5 mm.

Elevation: Convex.



Consistency: Very soft.

Chromogenesis: Glistening, grayish white; surface colonies have a pearl-like luster.

Internal structure: Granular.

Edge: Lacerate.

11. *Filter paper broths*, 15 days. Paper attacked along the edge nearest surface of solution; in 20 days the paper is very completely disintegrated. The paper decomposed at about the same rate in solutions containing ammonium sulphate, potassium nitrate, peptone, or casein as the source of nitrogen.

### III. BIOCHEMICAL FEATURES.

12. *Dunham's solution*, 10 days. Ammonia produced; no nitrite produced.

13. *Starch nitrate solution*. Ammonia produced; nitrite produced.

14. *Peptone nitrite broth*, 10 days. No indol produced.

15. *Carbohydrate broths*, 12 days. No gas produced. Per cent of acidity (Fuller's Scale) with: Dextrose, 1.25; Lactose, .50; Saccharose, 1.10; Maltose, 1.20; Glycerine, .30; Mannite, .25; Starch, 1.50.

### SUMMARY OF SPECIFIC CHARACTERISTICS OF CELLULOSE-DISSOLVING BACTERIA

The detailed description of the cellulose-dissolving bacteria known today are scattered through several publications. In the identification of newly isolated forms or in comparing the specific characteristics of described forms, it is obviously desirable that the more important morphological and cultural features of the cellulose-dissolving organisms known at this time be brought together in such a way as to afford a ready comparison. The more important morphological and cultural features of the cellulose-dissolving bacteria are briefly summarized in Table II. The biochemical reactions of the different species are summarized in Table III.

### PROVISIONAL KEY FOR IDENTIFYING AND COMPARING SPECIES OF BACTERIA WHICH DISSOLVE CELLULOSE

In order to facilitate further the identification and classification of cellulose-dissolving bacteria a diagrammatic key has been prepared. In the preparation of a key of this character, it is desirable to use single diagnostic features by means of which the organisms may be separated into smaller and smaller groups until all species are finally separated from each other. In such an arrangement, it is obvious that the features used must have a high degree of constancy. In the preparation of the following key, only those features which have remained constant through a number of cultures have been used and it is believed that when the key is used in conjunction with the data presented in Tables II and III, it will be found of much help in separating a particular organism from its congeners or in assigning it a provisional place in a system of classification.

### IMPORTANCE OF CELLULOSE DESTRUCTION IN SOILS.

All organisms make up for the waste incurred in their vital activities by the consumption of chemical energy. This necessary energy is for the

TABLE II  
COMPARATIVE SUMMARY OF THE MORE IMPORTANT MORPHOLOGICAL AND CULTURAL FEATURES OF CELLULOSE-DISSOLVING BACTERIA

	Morphology				Cultural features							
	Dimensions in microns	Number of flagella	Spores	Involution forms	Beef agar		Broth clouded	Gelatin liquefied	Growth on potato	Litmus milk		
					Luxuriant growth	Yellow chiro-mogenesis				Milk reddened	Milk blueed	Coagulated or digested
<i>B. albidus</i>	1.0 x .4	1-3	—	—	—	—	—	—	—	+	—	—
<i>B. almus</i>	1.2 x .5	1-5	—	—	—	—	+	—	—	+	—	—
<i>B. amylolyticus</i> (28)	3.5 x .7	10-16	+	—	+	+	+	+	—	+	—	—
<i>B. aurogenus</i> (29)	1.4 x .4	1-3	—	—	+	+	+	+	+	+	—	+
<i>B. bibulus</i> (42)	1.3 x .4	1-4	—	—	—	—	+	+	+	+	—	—
<i>B. biazoteus</i> (29)	.8 x .5	1-3	—	—	+	+	+	+	+	+	—	—
<i>B. caesius</i> (29)	1.5 x .4	1-2	—	—	—	—	+	+	—	+	—	+
<i>B. cellaseus</i> (29)	1.2 x .5	1-3	—	—	—	—	+	+	—	+	—	—
<i>B. concitatus</i>	1.2 x .5	1-3	—	—	+	+	+	+	—	+	—	—
<i>B. cytasceus</i>	2.7 x .5	10-18	+	+	—	—	—	—	—	—	—	—
<i>B. desidiosus</i>	1.0 x .4	1-3	—	—	—	—	+	—	—	+	—	—
<i>B. festinus</i>	2.0 x .6	1-3	+	—	—	—	—	—	—	+	—	—
<i>B. galbus</i> (29)	1.0 x .4	1-3	—	—	+	+	+	+	—	+	—	—
<i>B. gelidus</i> (29)	1.2 x .4	1-3	—	—	+	—	+	—	+	+	—	+
<i>B. gilvus</i>	1.5 x .5	1-4	—	—	—	+	+	—	+	+	—	—
<i>B. imminutus</i>	1.5 x .2	1-5	+	+	—	—	—	—	—	—	—	—
<i>B. iugis</i>	1.4 x .4	1-3	—	—	—	—	+	+	+	+	—	—
<i>B. pusilus</i> (29)	1.1 x .6	1-3	—	—	—	—	+	+	—	+	—	—
<i>B. rossicus</i> (28)	1.2 x .3	1-5	—	+	+	+	+	+	+	—	+	—
<i>B. subalbus</i>	.8 x .4	1-3	—	—	+	—	+	—	—	+	—	—
<i>Bact. acidulum</i> (29)	1.0 x .3	—	—	—	—	—	—	—	—	+	—	—
<i>Bact. castigatum</i>	1.2 x .4	—	—	—	+	—	+	—	—	+	—	—
<i>Bact. fimi</i> (42)	.9 x .4	—	—	—	—	—	+	+	+	+	—	—
<i>Bact. flavigenum</i> (28)	1.0 x .4	—	—	—	+	+	+	+	+	+	—	—
<i>Bact. idoneum</i>	1.5 x .5	—	—	—	—	+	+	+	+	+	—	—
<i>Bact. liquatum</i> (42)	1.7 x .4	—	—	—	+	+	+	+	+	+	—	—
<i>Bact. lucrosum</i>	1.3 x .4	—	—	—	+	—	+	—	—	—	—	—
<i>Bact. paludosum</i>	1.5 x .4	—	+	—	+	—	+	+	+	+	—	—
<i>Bact. udum</i> (29)	1.5 x .5	—	—	—	+	—	+	+	+	+	—	—
<i>Ps. arguta</i>	.8 x .3	1-2	—	—	—	—	+	—	—	+	—	—
<i>Ps. effusa</i> (29)	1.7 x .4	1-6	—	—	+	+	+	+	+	—	+	+
<i>Ps. minuscula</i>	.9 x .5	1-2	—	—	+	—	+	—	—	+	—	—
<i>Ps. mira</i>	1.6 x .4	1	—	—	+	—	+	—	+	—	+	—
<i>Ps. perlurida</i> (29)	1.0 x .4	1-3	—	—	+	—	+	+	+	+	—	+
<i>Ps. subcreta</i> (42)	1.4 x .4	1-5	—	—	—	—	—	—	—	—	—	—
<i>Ps. tralucida</i> (29)	1.2 x .6	1-2	—	—	—	—	+	—	—	+	—	—

most part derived from the oxidation of carbon. Green plants through the agency of their chlorophyll have the power of utilizing the radiant energy of the sunlight to decompose the carbon dioxide of the air and use it in their metabolic processes. These plants receive thereby not only the necessary energy for their own life, but store up an enormous quantity of potential energy upon which animals and those plants which do not contain chlorophyll are largely dependent. Moreover, the successful growth

**TABLE III**  
**COMPARATIVE SUMMARY OF THE BIOCHEMICAL FEATURES OF\***  
**CELLULOSE-DISSOLVING BACTERIA**

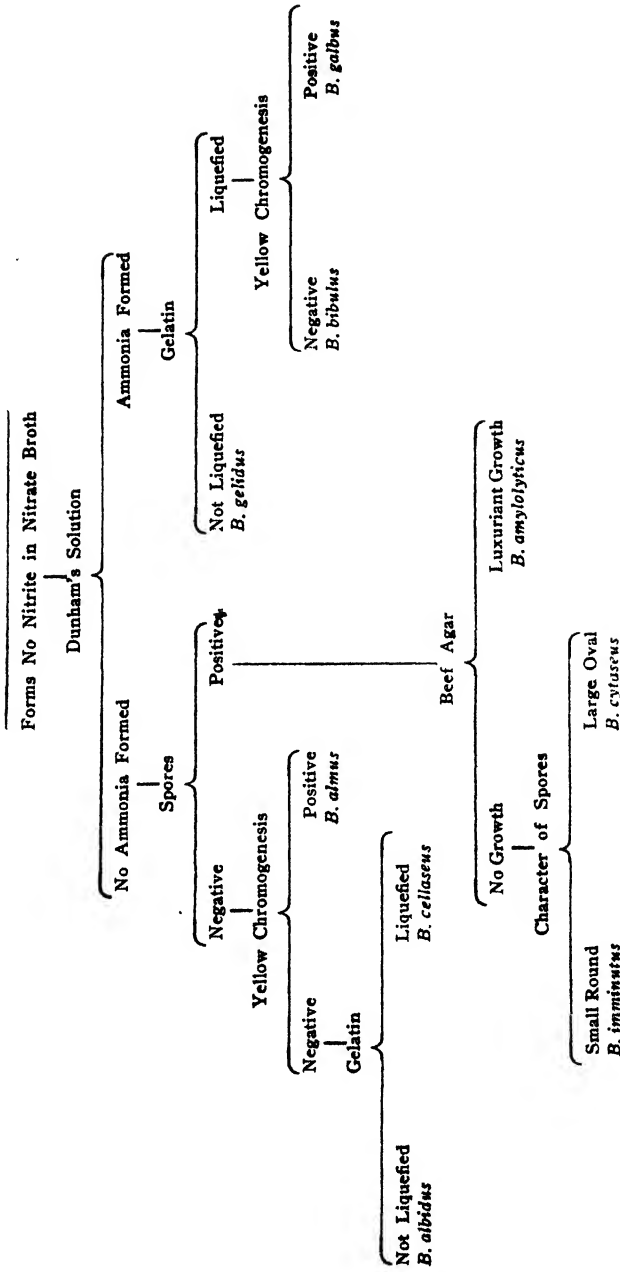
	Dunham's solution		Nitrate solution		Indol	Per cent acid produced in 12 days at 30° C.						
	Ammonia	Nitrite	Ammonia	Nitrite		Dextrose	Lactose	Saccharose	Maltose	Glycerine	Mannite	Starch
<i>B. albidus</i>	—	—	—	—	—	0.50	0.20	0.10	0.10	0.10	0.10	0.10
<i>B. almus</i>	—	—	—	—	—	1.30	0.80	1.00	1.20	0.40	0.00	0.60
<i>B. amylolyticus</i> (28)	—	—	—	—	—	0.90	0.90	0.90	0.80	0.90	0.90	1.40
<i>B. aurogenus</i> (29)	+	+	+	+	—	1.80	1.40	1.40	1.20	0.70	0.00	1.60
<i>B. bibulus</i> (42)	+	+	—	—	+	1.80	1.30	1.50	1.50	0.40	1.20	2.00
<i>B. biazoteus</i> (29)	—	+	—	+	—	2.00	1.10	1.00	0.90	0.50	0.00	1.50
<i>B. caesiue</i> (29)	+	+	+	+	—	1.90	1.50	1.40	1.10	0.50	0.20	1.40
<i>B. cellaseus</i> (29)	—	+	—	—	—	1.40	0.40	1.40	0.80	0.30	1.10	1.20
<i>B. concitatus</i>	—	—	—	+	+	1.80	0.85	1.30	1.30	0.45	0.00	1.35
<i>B. cytaseus</i>	—	—	—	—	—	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>B. desidiosus</i>	—	+	—	+	+	0.80	0.10	0.00	0.60	0.00	0.00	0.20
<i>B. festinus</i>	—	—	—	+	+	0.50	0.40	0.00	0.65	0.05	0.00	0.60
<i>B. galbus</i> (29)	+	+	—	—	+	1.40	1.30	1.20	1.30	1.20	0.00	1.30
<i>B. gelidus</i>	+	+	—	—	—	1.20	1.20	0.80	1.20	0.40	0.00	1.40
<i>B. gilvus</i>	+	+	—	+	+	1.20	0.75	0.80	1.00	0.40	0.00	1.00
<i>B. imminutus</i>	—	—	—	—	—	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>B. iugis</i>	+	+	—	+	—	0.80	1.10	1.60	1.55	0.45	0.20	1.50
<i>B. pusilius</i> (29)	+	+	—	+	—	1.50	1.40	1.60	1.40	0.50	0.00	1.50
<i>B. rossicus</i> (28)	+	—	—	+	—	-1.0	-1.4	-1.4	-1.6	-1.4	-1.5	-1.2
<i>B. subalbus</i>	+	+	—	+	—	1.60	1.00	1.40	1.20	0.70	0.20	1.40
<i>Bact. acidulum</i> (29)	—	—	—	—	—	0.40	0.30	0.30	0.50	0.00	0.00	0.00
<i>Bact. castigatum</i>	—	+	—	—	—	1.50	1.10	1.00	1.45	0.55	0.00	1.40
<i>Bact. fimi</i> (42)	+	+	—	+	+	1.60	0.90	1.60	1.40	0.80	0.00	1.60
<i>Bact. flavigenum</i> (28)	—	+	—	+	—	1.00	0.90	0.70	0.90	0.30	0.10	1.40
<i>Bact. idoneum</i>	—	+	—	+	—	1.60	1.20	1.20	1.40	0.70	0.00	1.40
<i>Bact. liquatum</i> (42)	+	—	—	+	+	1.30	1.00	1.30	1.20	0.20	0.00	1.40
<i>Bact. lucrosus</i>	—	+	—	—	—	0.20	0.10	0.00	0.15	0.00	0.05	0.15
<i>Bact. paludosum</i>	—	+	—	—	+	1.10	0.80	1.00	1.20	0.40	0.05	1.20
<i>Bact. udum</i> (29)	—	+	+	+	—	1.40	1.30	1.40	1.30	0.00	0.00	1.40
<i>Ps. arguta</i>	—	+	—	—	—	0.30	0.10	0.00	0.20	0.00	0.00	0.30
<i>Ps. effusa</i> (29)	+	—	—	+	—	2.10	-0.50	-0.70	0.60	0.30	0.20	1.20
<i>Ps. minuscula</i>	—	+	—	+	+	1.20	1.10	1.00	1.10	0.00	0.00	0.90
<i>Ps. mira</i>	+	—	—	+	—	1.25	0.50	1.10	1.20	0.30	0.25	1.50
<i>Ps. Perlurida</i> (29)	+	—	—	—	—	1.80	1.50	1.50	1.20	0.60	1.50	2.00
<i>Ps. subcreta</i> (42)	—	—	—	—	—	0.60	0.50	0.10	0.50	0.00	0.00	0.60
<i>Ps. tralucida</i> (29)	—	+	—	+	—	1.30	0.70	1.60	1.00	0.40	0.20	1.60

of future generations of green plants is largely controlled by the liberation of this large store of potential energy through decomposition processes in the soil.

It is well known that through the agency of microorganisms, vegetable matter is gradually transformed into the complex mixtures ordinarily known as humus. In all cultivated soils, it is important to replenish from time to time the organic matter in the soil by the application of stable manure, green manure, etc. In semi-arid soils where the growth

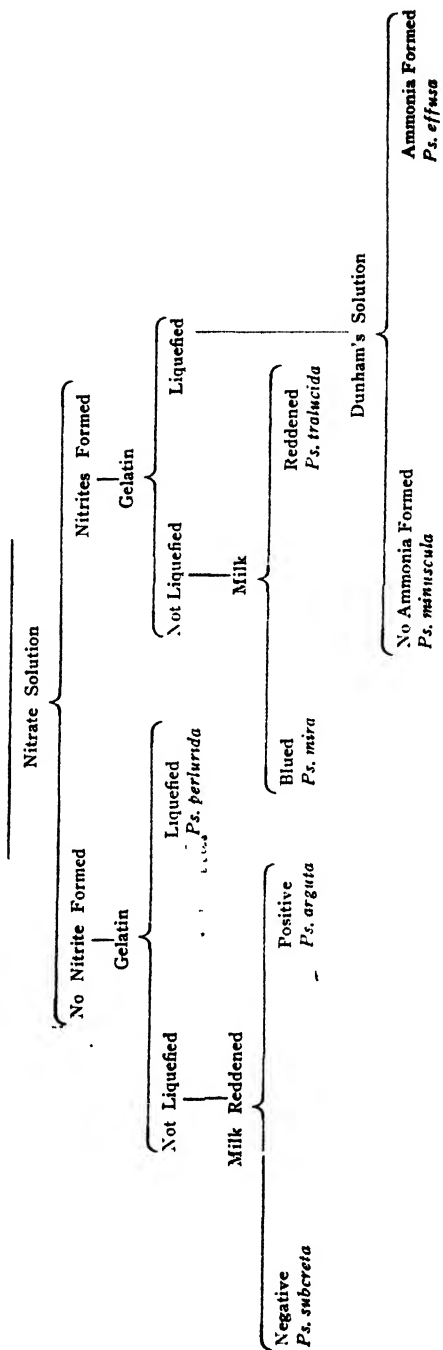


PROVISIONAL KEY FOR IDENTIFYING AND COMPARING SPECIES OF BACTERIA WHICH DISSOLVE CELLULOSE  
GENUS *BACILLUS*, Part II





PROVISIONAL KEY FOR IDENTIFYING AND COMPARING SPECIES OF BACTERIA WHICH DISSOLVE CELLULOSE  
GENUS *PSEUDOMONAS*

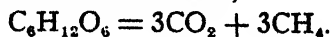


of native vegetation has been limited by the meager rainfall, the humus content of the virgin soil may be as low as 0.30 per cent or even less. When such soils are brought under intensive cultivation by means of irrigation, the scarcity of humus soon manifests itself by the development of injurious changes in the tilling qualities of the land. Many such lands soon fail to give satisfactory crops or respond to the application of commercial fertilizers unless the supply of organic matter is maintained by liberal applications of barnyard manure, green manures, etc.

As the larger part of carbonaceous matter added to soils in plant residues, stable manure, etc. is cellulose,—the gradual decomposition of the cellulose in soils in association with the nitrogenous compounds must play a very prominent rôle not only in maintaining the humus content of soils, but in securing the proper development of the many important biological processes. The humus content of the soil is considered by many to serve as the depository of the insoluble nitrogen of the soil which constitutes the reserve supply for crops. It is probable but not certain that this insoluble nitrogen through the process of nitrification furnishes the main nitrogen supply to plants. The fixation of atmospheric nitrogen in the soil is dependent upon the development of micro-organisms which requires large quantities of organic carbon as food. During recent years, investigations by Koch (34), Pringsheim (63), and McBeth (42) have shown that cellulose may serve as a valuable source of energy for these organisms. However, cellulose is an extremely inert compound and the carbon contained therein can be utilized by the nitrogen fixing bacteria only after the cellulose has been converted into less refractory compounds by the cellulose-dissolving bacteria. It is obvious, therefore, that the work performed by these organisms is of fundamental importance in releasing the great store of energy locked up in cellulose. In view of the fact that the cellulose added to the soil represents a large amount of potential energy, the value of which depends upon the nature of the compounds formed in its decomposition, it becomes quite important to inquire into the nature of the compounds produced by the cellulose-dissolving bacteria. Earlier investigations by Popoff (61), Toppeiner (78), Hoppe-Seyler (25), Gayon (15), Deherain (13), Schloesing (74), Van Senus (76), Omeliansky (50), and others seemed to indicate that cellulose undergoes a direct gaseous fermentation in which a very large percentage of the carbon is converted into carbon dioxide and methane. Hoppe-Seyler was of the opinion that cellulose was dissolved according to the following formula:

(1) The hydration of the cellulose with the formation of a hexose,  
$$\text{C}_6\text{H}_{10}\text{O}_5 + \text{H}_2\text{O} = \text{C}_6\text{H}_{12}\text{O}_6; \text{ and}$$

(2) The destruction of the carbohydrate with the formation of equal quantities of carbon dioxide and methane,



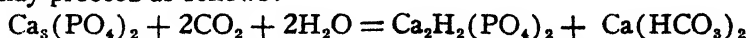


If cellulose undergoes a direct gaseous fermentation in which a large part or all of the carbon is returned to the air in the first decomposition processes, the addition of cellulose to the soil would undoubtedly be of far less value than if the decomposition products formed by the cellulose-dissolving bacteria were non-volatile and remain in the soil, where they may assist in maintaining the humus content or may serve as a source of energy for important groups of bacteria, such as the nitrogen fixing organisms.

It is well known that fermentation processes in the soil resulting in a decomposition of the organic matter may give rise to large quantities of  $\text{CO}_2$  and  $\text{CH}_4$ . However, we have been unable to show that these compounds are due to the activity of cellulose-dissolving bacteria. None of the cellulose-dissolving forms studied in our investigations give rise to gaseous products in cellulose or sugar solutions in which they make a luxuriant growth. Under natural conditions the compounds formed by the cellulose-dissolving bacteria will of course be seized upon by a host of other microorganisms and split up into simple compounds. In some soils the destruction may be extremely rapid and complete, resulting in the formation of little humus; under such conditions a very large percentage of the carbon in the cellulose is quickly liberated as  $\text{CO}_2$ . However, the  $\text{CO}_2$  formed is presumably due in all cases to secondary fermentations by the action of the organisms upon the products produced by the cellulose-dissolving organism. Likewise, the organic acids noted by early investigators were, for the most part at least, presumably due to secondary fermentation and not to the action of the cellulose-dissolving forms.

The influence of the products of bacterial activity in rendering soluble various essential mineral constituents of the soil has come to be recognized as of considerable importance in maintaining the fertility of soils. It would seem that the insoluble compounds of potassium, phosphorus, magnesium, calcium, iron, sulphur, and even silicon may be rendered soluble through the production of carbon dioxide and organic acids which result from the decomposition of cellulose and other organic matter in soils. It is well known that limestones are quickly dissolved by carbonated waters, even granite and rocks related to it are attacked because of the feldspar minerals which contain potash, sodium and calcium together with aluminum. The results of this action would seem to be highly important in many western soils as the liberation of the aluminum results in the formation of clay which has an important influence on the physical condition of the soil, while the potassium is one of the essential nutrients of plant growth.

Phosphoric acid is so tenaciously held by most soils that ordinary leaching of the soil due to natural rainfall or irrigation would seem to bring very small amounts of this valuable substance into solution. The action of carbon dioxide upon the insoluble phosphorus compounds of the soil may proceed as follows:



A large portion of the  $\text{CO}_2$  resulting from the decomposition of cellulose or other carbonaceous materials in soils is ultimately returned to the atmosphere where it may be used over and over again in the manufacture of sugar, starches, cellulose, etc. in new generations of plants. If it were not for the activity of cellulose-dissolving organisms in the soil developing in association with gas producing organisms, the cycle of change to which carbon is subject would soon come to a standstill and the carbon supply of plants soon be depleted.

The importance of cellulose destruction in soil may then be summarized as follows:

1. The decomposition of cellulose under proper soil conditions and in association with the nitrogenous compounds of plant tissues makes possible the maintenance of the soil humus which is so essential in maintaining the proper tilling qualities of the land.
2. The cellulose added to the soil represents a large amount of potential energy which must have a marked stimulating effect on nitrogen fixation and many other important biological processes going on in the soil.
3. The decomposition of cellulose in soils, under proper conditions, results in the formation of large quantities of carbon dioxide. The action of carbonic acid in rendering available various mineral constituents of the soil is recognized as an important factor in the maintenance of soil fertility.
4. Through the decomposition processes, the carbon locked up in the cellulose is ultimately returned to the atmosphere, thus maintaining the carbon cycle and rendering the carbon supply for plants inexhaustible.

#### SUMMARY

1. The cellulose agar plate method is the most satisfactory for isolating pure strains of bacteria, filamentous fungi or Actinomycetes which have the power of dissolving cellulose.
2. In the preparation of precipitated cellulose for cellulose agar, the copper-ammonium-cellulose solution as well as the acid used should be very dilute. If either of the solutions are too concentrated, the precipitate is likely to be coarse, which not only makes it difficult to wash, but unsatisfactory for the preparation of culture media. A uniformly fine cellulose precipitate can be secured by diluting one part of the copper-ammonium-cellulose solution with forty parts of water and mixing with

a dilute hydrochloric acid solution, prepared by adding one part of concentrated acid to twenty parts of water.

3. Cellulose agar can be prepared from the cellulose in plant tissues by grinding the dry plant substances to a flour and isolating the cellulose in a pure state from the finely ground substance. Cellulose prepared in this way is quite as satisfactory for the preparation of cellulose agar as that prepared from filter paper in the ordinary way.

4. Twenty-five species of cellulose-dissolving bacteria have been grown on culture media containing cellulose prepared from alfalfa flour. All of the organisms plated to this medium dissolved the cellulose as readily as that prepared from filter paper.

5. All of the cellulose-dissolving organisms studied develop most rapidly in the presence of air, although more or less growth can be secured under anaerobic conditions.

6. Most of the cellulose-destroying bacteria grow well upon ordinary culture media. A few forms do not grow upon ordinary culture media, but only upon media containing cellulose.

7. The cellulose-dissolving bacteria assimilate nitrogen from organic as well as inorganic nitrogenous compounds. Many forms destroy cellulose rapidly when the culture medium contains nitrogen in the form of peptone, ammonium sulphate, potassium nitrate or casein. Peptone appears to be most favorable for the largest number of species, while casein is usually least favorable of the nitrogen compounds tested.

8. The quantity of acid formed in carbohydrate broths, in 12 days at 30° C. usually amounts to from 1 to 2 per cent on Fuller's scale, with dextrose, lactose, maltose, saccharose, and starch. The per cent of acidity in mannite and glycerine solutions is usually less than 1 per cent and in many instances no acid is formed from these substances.

9. Many species of cellulose-dissolving bacteria produce a small quantity of nitrite in Dunham's solution. The nitrite is presumably formed from the peptone. A starch nitrate broth free from peptone has therefore been used instead of the standard nitrate broth for determining the nitrate reducing power of these organisms.

10. Filamentous fungi play a much more important rôle in the destruction of cellulose in the humid soils of the eastern part of the United States than in the semi-arid soils of southern California.

11. Species of cellulose-dissolving *Actinomyces* have a wide distribution in soils and are unquestionably a factor in the destruction of cellulose in nature.

12. The very rapid destruction of cellulose which occurs in many soils of southern California is probably due to favorable climatic and cultural conditions which make possible the rapid development of the cellulose-dissolving organisms rather than to the unusually active nature of the cellulose-dissolving soil flora.

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## THE INFLUENCE OF LIME ON THE YIELD AND NITROGEN CONTENT OF CORN<sup>1</sup>

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The practice of using lime in some one or another of its forms, for agricultural purposes, is very old. European countries recognized the value of such materials centuries ago, even before the Christian Era. In this country their value was recognized by individuals here and there throughout the first half of the nineteenth century and even earlier, but it is only within the last twenty or thirty years that the use of these materials has become very general.

Used in a judicious manner there is no question as to the beneficial effects of lime, over widely separated areas of the country and on a great variety of soils. There are comparatively few crops grown in the older sections of the country, where the soils are not naturally well supplied with carbonate of lime, that do not respond to periodic applications of lime.

Even soils of limestone origin, under long continued cultivation, may become so exhausted of their lime compounds as to respond to lime treatment. There is not always agreement as to the function of the lime in connection with the soil and the growth of the plant, but there is a very general agreement as to the final result in crop yield and generally improved soil conditions.

It is probable that the cases are rare where applications of lime are required as a source of actual food for the plant. It is quite certain, on the other hand, that it has much to do with the physical condition of the soil. It is likewise true that it has an effect upon the mineral and vegetable matter of the soil and upon soil organisms. Not the least important of these effects is the part which it plays in making available plant food out of resistant organic matter. This is undoubtedly what does occur when lime is applied to soils that have become acid, but still contain a considerable supply of organic residues. Thus lime becomes an agent in improving soils deficient in available nitrogen, in this way as well as by making the conditions more favorable for those plants which, by the aid of certain organisms, store up the nitrogen of the air.

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<sup>2</sup> The field work in connection with this experiment was under the direction of Mr. L. F. Merrill.

It is not intended here to enter into an extended bibliography touching the subject of lime, but rather to cite a few of the more important experiments where lime has been used in connection with the corn crop.

In a report issued in 1894, Wheeler, Towar and Tucker (16) state that with 3 tons of air-slaked lime to the acre corn was injured. With smaller quantities of lime corn was slightly benefited or uninjured.

Patterson (13) found that applications of oyster-shell lime increased the yield of corn. In a later report (14) the same author states that in a rotation of corn, wheat, and timothy and clover on run down sandy loam naturally well drained, the limed plots gave larger yields than the unlimed plots, the average net return being \$4.50 an acre per year.

Discussing the use of ground limestone for acid soils in Illinois, Hopkins (3) reports that as the average of twenty tests on different experiment fields, the yield of corn was increased 6.6 bushels and as an average of eighteen tests the yield of wheat was increased 4.8 bushels per acre, both of these crops being grown in rotation with legume crops on both limed and unlimed land. Hopkins recommends the use of ground limestone when it can be obtained.

Hunt (4), summarizing the results of a series of fertilizer experiments on a clay loam soil, of limestone origin, which have been carried on for twenty-five years, says, "An acid condition, proving especially injurious in later years to the corn and clover, resulted from the continued application of sulfate of ammonia. The addition of 4000 pounds of quick lime applied once in four years to the plots receiving no fertilizer has caused a decrease in yield, but when applied in connection with 6 tons of barnyard manure, the products produced were equal to those produced by an application of 10 tons of manure without lime."

Lyon and Morgan (11) discussing the effect of fertilizers applied to timothy and the corn crop following it, say, "Lime had the effect of rendering available plant nutrients in the soil but did not increase the efficiency of the fertilizers." Since the percentage of increase was greater when fertilizers were not applied, the authors regard its beneficial effect as due to the direct liberation of plant-food rather than to its neutralizing or other action.

Mooers and Robert (12), reporting on experiments conducted on a light brown-colored silt loam and a gray-colored "crayfishy" type, say, "In studying the effect of burnt lime and ground limestone applied at the rate of 2000 pounds and 4000 pounds respectively, per acre, it was observed that an increased yield of corn, oats and red clover followed the applications of lime on both types of soil." They claim that the results from the two kinds of lime were very similar, the ground limestone, however, being slightly superior.

In Bulletin 279 of the Ohio Agricultural Experiment Station, Thorne (15) reports the average results secured over a period of 12 years in a 5-year rotation of corn, oats, wheat, clover and timothy, where lime and ground limestone were used on certain of the plots while other plots received no lime. The experiments were conducted on a light silty clay which had previously been subjected to an exhaustive system of farming. In a review of this work reported in Vol. 1, No. 1 of the monthly bulletin of the Ohio Station, Thorne gives a condensed table of yields and draws attention to the way in which the corn has followed the lime. "In 1900 the yield on the west end of Section E, just limed, was 8 bushels more than on the east end. In 1905, the liming was transferred to the east end and the yield was nearly 12 bushels greater than it had been on that end in 1900 while the west end limed in 1900, but not in 1905 still showed the effect of the liming although its yield was several bushels below that of the newly limed east end. Were the corn the only crop benefited by liming the cost would outweigh the gain; but the average results for the entire rotation showed that there has been a gain on the unfertilized land of 4.76 bushels of oats, 2.75 bushels of wheat, 494 pounds of clover hay and 641 pounds of timothy hay in the average of the crops following the corn, the whole having a total value of \$13.82, if we value corn at half a dollar per bushel, oats at one-third of a dollar, wheat at 90 cents and hay at \$10 per ton. The average cost of liming has been about \$5 per acre; so there has been an ample margin of profit.

In lime tests in various parts of Alabama (1) the yield of corn was increased by the use of lime in all but three of the experiments. The average increase was 11 per cent.

Under a discussion of lime for the tidewater section of Virginia, Ellett (2) says, "Stable manure and lime increased the yield 100 per cent above commercial fertilizers alone. Lime increased the yield 39 per cent above commercial fertilizers and manure."

In a bulletin on corn experiments, Williams and Welton (18) say, "On such acid soils as are found on the station farm at Wooster, 1 ton of burned lime, or 2 tons of ground limestone, applied once in 5 years, has increased the yield of corn on an average 7.35 bushels per acre on the fertilized plots reported in Table III, and 8.25 bushels per acre on the unfertilized plots. Taking into consideration all the crops of the rotation the application of lime has been worth, on the average \$14.21 per acre per rotation. The cost of the lime has been \$5.00."

Lipman and Blair (10) have shown that lime increases the yield of dry matter (forage) and also the amount of nitrogen recovered in the crop, when corn is grown in cylinders in a regular 5-year rotation, and also when it is grown as a residual crop in the rotation.

When lime was used with a complete fertilizer, Williams, Kilgore and Russell (17) found that "On an average taking the results of both fields together, there was an increase due to the lime above the cost of the lime to the value of \$2.89 per acre on the basis of corn alone and of \$4.52 on the basis of corn and stover together." When used without commercial fertilizers the lime was not profitable.

The work here reported constitutes a part of a regular 5-year rotation of corn, oats, oats, wheat, and timothy that is being conducted at the New Jersey Agricultural Experiment Station, the results of the first five years having been published (6). The corn crop here referred to was the first crop in the second rotation.

The work is suggestive and raises questions which have not been fully touched upon. For example, certain of the data presented in Table I will appear more intelligible when considered in the light of the completed rotation. A discussion of the causes which may account for the differences in the recovery and availability of nitrogen as observed, is reserved for a more detailed consideration in another paper.

The experiment was planned primarily to study the availability of nitrogen in different nitrogenous materials, but since there are two sections which receive similar treatment with respect of nitrogenous constituents, it was possible to lime one section while the other section has, all the while, remained unlimed. The lime treatment has been 1 ton of ground limestone per acre in 1908 preceeding the first crop of corn, and 2 tons per acre in 1913, preceeding the second crop of corn.

The soil was described in the publication just referred to and it is necessary here only to say that it is a loam which contains a considerable portion of small pebbles scattered through it. Prior to the beginning of this experiment the land had been neglected for a number of years.

The lime requirement of the soil was not determined when the work was begun, but that the soil was originally acid is indicated by the fact that no lime had been applied in recent years and by the further fact that notwithstanding the application of 1 ton of ground limestone per acre in 1908 the soil on all the plots was decidedly acid at the end of the first five years, no plot requiring less than 1000 pounds of lime ( $\text{CaO}$ ) per acre to neutralize the acidity to a depth of about seven inches.

The influence of the first application of lime seems, however, not to have entirely disappeared, for volunteer red clover appeared on nearly all the plots following the timothy crop in 1912, and as elsewhere (6) noted there was nearly twice as much clover taken from the limed plots as from the unlimed plots. Furthermore, that from the limed plots contained, in the dry matter, about .5 per cent more nitrogen than that from the unlimed plots. Table I indicates the fertilizer treatment the different plots have received. From this it will be noted that six of the plots have

no nitrogen applied to them and that 1 A and 1 B, and 7 A and 7 B receive neither nitrogen nor mineral fertilizers. These facts in part account for the low average yields for the 1908 and 1913 crops.

## CROP OF 1908

Since this work has already been published it is necessary here to give only a brief summary of the figures. A comparison of the limed and unlimed sections gives the following average yields per acre.

	Limed	Unlimed
Grain.....	46.00	39 31 bu. per acre
Stover.....	3466.20	2948 60 lbs. per acre
Nitrogen removed in crop.....	Grain 37.52 Stover 27.78	32.30 lbs. per acre 23.22 lbs. per acre
Nitrogen in dry matter.....	Grain 1 45 Stover 796	1.46 per cent .79 per cent
Nitrogen recovered in crop.....	33 23	12 80 per cent

From these figures it will be observed that the limed section gave higher results than the unlimed in all cases except the percentage of nitrogen in the dry matter, grain and stover, which is practically the same in both sections. The increase in yield of grain alone would more than pay for the lime that was used for the entire rotation. Furthermore, it is entirely possible that a heavier application of lime in 1908 might have made even a greater difference between the two sections, for as already noted the first application of ground limestone was not sufficient to keep the soil neutral throughout the rotation and it has already been shown by many experiments conducted in this country and also abroad, that the remaining crops used in this rotation normally respond to lime treatment, whereas in this case the yields of oats, wheat and timothy were just about as good on the unlimed as on the limed sections.

That the increased yield of corn in the limed plots was due in part, at least, to a larger supply of available nitrogen resulting from a more thorough nitrification of the soil organic matter, is made clear by a comparison of the yields on certain plots which received an extra supply of nitrogenous materials, with the yield from certain other plots which received a limited supply of nitrogen. It will be noted, for example, that plots 5 and 6, and 18 and 20 in both sections received an extra supply of nitrogen in the form of manure or nitrate of soda or both, and on these plots the lime did not result in any greater average increase in yield than it did on plot 15 B for example which received only 49 pounds of nitrogen. This probably means that an excess of available nitrogen was applied to plots 5, 6, 18 and 20 and therefore that which was made available by the lime applied to these plots in the B section did not influence the crop yield. Furthermore, the basic materials furnished by the manure, and the soda furnished by the nitrate of soda would tend to obliterate the effect of the lime.



## CROP OF 1913

The differences in the yield for this year are even more pronounced than in the crop of 1908. However, it will be remembered that preceeding the 1913 crop the plots in the limed section received ground limestone at the rate of 2 tons per acre. The curves in figure I, show the lime requirement of the various plots before this application was made.

The yield of dry matter and other data for the second rotation are set forth in Table I.

Referring to the column marked "Nitrogen Applied" it will be noted that in the case of certain plots 5, 6, 16, 17, 18 and 20, both sections, much more nitrogen was applied than was applied to the other plots which call for a nitrogen treatment. This is due to the fact that for the former plots the plan calls for a definite amount of nitrogenous fertilizer, as manure or other organic material, rather than a definite amount of nitrogen, as in a majority of the nitrogen treated plots.

## THE YIELD OF DRY MATTER

With the exception of plots 1, 5, and 6, the limed sections gave the largest yield of grain, and with the exception of plots 1, 2, 3, 6 and 18, likewise the largest yield of stalks. In the case of 5, 6, and 18, the explanation for this seems to lie in the fact that for these plots, the manure and nitrate of soda furnished a sufficient amount of available nitrogen and also a certain amount of basic materials so that the crop was not dependent on the nitrogen that was made available by the lime. No definite reason is at present assigned for the failure of 1 B, 2 B and 3 B to yield as much grain as 1 A, 2 A and 3 A.

It is of interest to compare plots 17 A and 17 B. These plots received wheat straw or rye straw as a source of nitrogen. These materials are low in nitrogen, and besides, when used on an acid soil, they lie in that soil more or less inert very much like the organic matter that is already in the soil. On the other hand, when used in connection with some form of lime as in 17 B, they decompose much more rapidly, thus yielding available nitrogen. At the same time the lime acts on the soil organic matter also, making some of the nitrogen available. Thus the yield on 17 A is at the rate of 19.2 bushels of grain and 2000 pounds of stalks while the yield on 17 B is 46.4 bushels of grain and 2525 pounds of stalks per acre. Furthermore, the percentage of nitrogen in both grain and stalks from 17 B is higher than from 17 A.

The difference between 16 A and 16 B is not so marked for the reason that green alfalfa or alfalfa hay was used on these plots as a source of nitrogen. A given weight of this material not only furnishes more nitrogen than an equivalent weight of wheat or rye straw, but the nitrogen of the alfalfa is more available than that of the wheat straw or of the rye straw

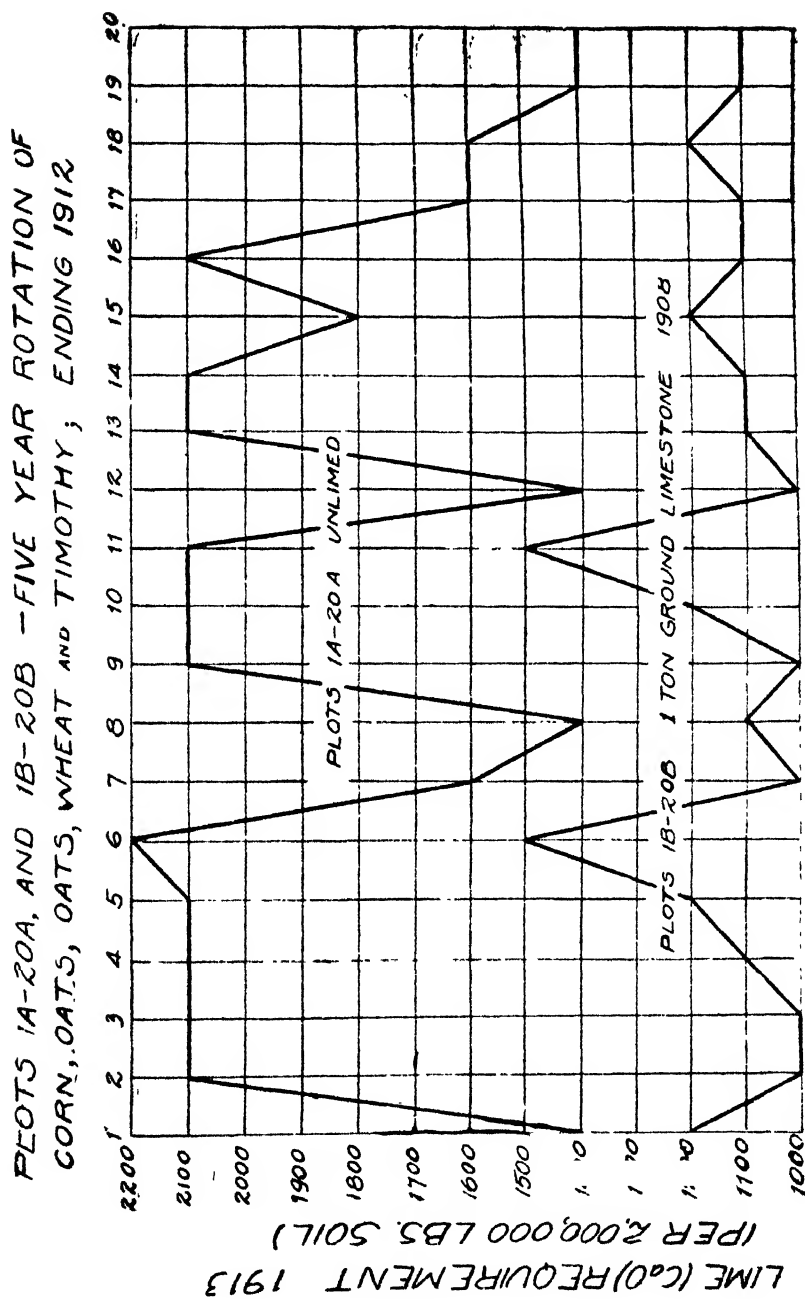
Fig 1.—Lime requirement of soil from unlimed and limed plots.<sup>1</sup><sup>1</sup> Taken from Bulletin No. 260, New Jersey Agricultural Experiment Station.



TABLE I—(Continued)  
YIELD OF DRY MATTER AND PERCENTAGE OF NITROGEN. LIMED SECTION 1913  
(Results calculated to acre basis)

No.	Treatment	Nitrogen applied, lbs.	Dry Matter			Per Cent Nitrogen			Total Nitrogen, lbs.	Increase Over Check			Per Cent Nitrogen Recovered
			Grain, bu.	Stalks, lb.	Cobs, lbs.	Grain	Stalks	Cobs		Grain, bu.	Stover, lbs.	Total N: trogen, lbs.	
1B	Nothing	.....	12.946	1375	150	1.600	.898	.464	24.644	.....	.....	.....	.....
2B	16 lbs. muriate of potash	.....	22.321	1425	175	1.462	1.026	.524	33.813	.....	.....	.....	.....
3B	12 lbs. acid phosphate	.....	16.518	1350	225	1.590	.957	.543	30.764	.....	.....	.....	.....
4B	Minerals only	.....	*27.679	*2350	*175	1.413	.789	.445	*39.810	.....	.....	.....	.....
5B	Minerals + 1600 lbs. cow manure	.....	173.47	38.839	2800	3.25	1.620	.947	63.105	15.847	625	27.138	15.64
6B	Minerals + 1600 lbs. horse manure	.....	215.84	29.464	1725	2.50	1.630	.967	504	6.472	.....	8.874	4.11
7B	Nothing	.....	28.125	1875	275	1.600	1.016	.415	45.391	*5.133	.....	*9.424	.....
8B	Minerals + 8 lbs. NaNO <sub>3</sub>	.....	24.50	36.161	2100	3.00	1.541	.996	494	53.603	.....	17.636	71.98
9B	Minerals + 16 lbs. NaNO <sub>3</sub>	.....	49.00	35.268	2300	3.50	1.600	.878	514	53.593	12.276	150	35.97
10B	Minerals + Ca(NO <sub>3</sub> ) <sub>2</sub>	.....	49.00	42.411	2650	3.25	1.531	.710	.405	56.491	19.419	475	41.88
11B	Minerals + (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	.....	49.00	45.536	2725	3.75	1.482	.878	.445	63.386	22.544	600	55.94
12B	Minerals + CaCN <sub>2</sub>	.....	49.00	39.732	2850	3.25	1.581	.711	.425	56.822	16.740	675	42.55
13B	Minerals + dried blood	.....	49.00	39.732	2700	3.25	1.541	.781	.454	56.951	16.740	525	42.61
14B	Minerals + dried fish	.....	49.00	40.179	2675	3.25	1.541	.701	.415	54.774	17.187	500	38.38
15B	Minerals + 16 lbs. NaNO <sub>3</sub>	.....	49.00	41.964	2425	3.50	1.571	1.045	.385	63.608	18.972	275	56.41
16B	Minerals + 800 lbs. green alfalfa	.....	147.30	39.286	2575	3.50	1.610	1.164	.425	66.881	16.294	425	30.914
17B	Minerals + 800 lbs. green wheat or rye	.....	92.12	46.429	2525	3.75	1.719	1.026	.514	72.529	23.437	400	20.99
18B	Minerals + 1600 lbs. cow manure and 16 lbs. NaNO <sub>3</sub>	.....	222.46	52.679	2625	4.25	1.590	.967	.425	74.095	29.687	550	36.562
19B	Minerals only	.....	*18.304	*2300	*175	1.334	.631	.534	*32.123	.....	.....	.....	.....
20B	Minerals + 800 lbs. green wheat or rye and 16 lbs. NaNO <sub>3</sub>	.....	141.10	43.304	400	1.699	.986	.435	68.924	20.312	525	32.957	23.36
Average		.....	34.844	2309	296.5	1.563	.904	.459	52.802	17.793	408.9	24.712	36.189

\* Nos. 4 and 19 averaged for check. Stalks + Cobs = Stover.

\* Minerals = 32 lbs. acid phosphate and 16 lbs. muriate of potash.

and the crop is supplied with nitrogen even though lime is not present to act upon resistant organic matter. Plots 7 A and 7 B received no nitrogenous fertilizers and 8 A and 8 B only a half-portion of the nitrate of soda, but 7 B and 8 B gave much larger yields than 7 A and 8 A for the reason that the lime caused a fair amount of the nitrogen of the inert soil organic matter to be brought into an available form. In the case of plot 7B which did not receive minerals, the increase is no doubt due in part to potash and phosphoric acid that were made available by the lime.

Attention may also be called to the yields on plots 11 A and 11 B, the former giving 23.2 bushels of grain and 1650 pounds of stalks and the latter 45.5 bushels of grain and 2725 pounds of stalks per acre. On plot 11 A the soil had become so acid from the continued use of ammonium sulphate that the yield was very much depressed. The lime used on plot 11 B corrected the acidity and as a result the yield was almost doubled.

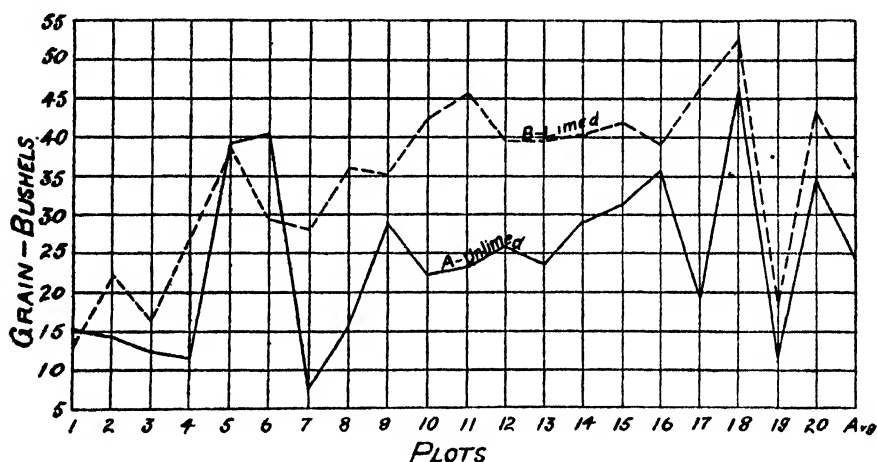


Fig. 2.—The influence of lime on the yield of corn, 1913.  
(Calculated to acre basis.)

The combination of manure, nitrate of soda and lime used on 18 B resulted in a yield of 52.7 bushels of shelled corn as against 46.4 bushels on 18 A. These were maximum yields for the limed and unlimed plots, respectively, and emphasize the value of the two forms of nitrogen, that is, a slowly available material with much organic matter, and the concentrated material that is quickly available. It is very certain that this combination furnishes a large excess of nitrogen and certainly the expense of such treatment is altogether out of proportion to the returns. In this case the application of nitrogen amounted to 222.5 pounds per acre whereas with 49 pounds of nitrogen in the form of commercial nitrogenous materials a yield of about 40 bushels of corn per acre was secured.

It is true that with commercial materials only, the supply of organic matter in the soil would not be kept up indefinitely, but in an approved

rotation this can be taken care of largely by the introduction of legumes as green manure crops.

The yield of shelled corn on the two sections is indicated by curves in figure 2.

#### PERCENTAGE OF NITROGEN IN THE DRY MATTER

With a few exceptions the percentage of nitrogen in the grain and stalks is higher for the plots which received the large application of nitrogen than it is for those plots that received the standard application. It is true also that the average percentage of nitrogen in the dry matter in both the grain and stalks from the plots that received no nitrogen treatment is less than it is in the dry matter from the plots that received nitrogen. It is likewise true with reference to grain and stalks, that the average percentage of nitrogen in the dry matter from all plots in the limed section is greater than the average from all plots in the unlimed section.

This effort of the plant to utilize available nitrogen when present has been referred to in earlier publications (5, 10). The fact that the dry matter from the limed plots contains a higher proportion of nitrogen than that from the unlimed plots seems to be confirmatory evidence that the lime plays a part in making available the nitrogen of the inert soil organic matter. Reference has already been made to the higher nitrogen content of clover grown on limed plots. This likewise is undoubtedly a case of more available nitrogen, but with the clover the additional nitrogen is probably drawn largely from the air, whereas in the case of the grain it must come from the soil or from applied fertilizers.

In experiments with soybeans (8, 9) on limed and unlimed plots it has been shown that the shelled beans grown on the former contain about 0.5 per cent more nitrogen than those grown on the latter, and this applies to an average obtained from some six or seven varieties. Here the lime aids those organisms that live in the plant and store up nitrogen from the air, and likewise those that live in the soil and convert dead organic matter into plant food, while in the case of the corn it probably aids the latter only. Similar observations have been made with reference to oats and peas, vetch, oats, lima beans (seed), cowpeas (hay), and timothy and clover (7, p 442-451; 8, p. 237), with the exception that the increase in most cases is not so great as with soybeans. It thus appears that lime aids in the utilization, by the crop, of a greater amount of nitrogen in the case of both leguminous and non-leguminous crops. However, with the latter this extra amount of nitrogen should be furnished by the introduction, in the rotation, of leguminous crops rather than by the purchase of nitrogenous materials.

## TOTAL NITROGEN RECOVERED

Since there is not a great variation in the percentage of nitrogen in the dry matter, the total nitrogen recovered in the crop will depend largely on the variation in the yields of dry matter, and as the yields were greater on the limed than on the unlimed sections, it naturally follows that the most nitrogen was recovered from the former. Of the plots that received nitrogen treatment, 6 B is the only exception to this, the yield on this plot being 44.84 pounds per acre as against 60.13 pounds on 6 A. No explanation appears for this. It received more nitrogen than 5 B and there seems no reason why it should not have yielded at least as much to the crop as 5 B.

The highest yield of nitrogen, 74.1 pounds per acre was on plot 18 B and the second highest, 72.5 pounds on 17 B. The latter is of especial interest since it is more than double the yield on 17 A, and shows in a striking manner, how lime makes the nitrogen of inert organic material—in this case rye straw—available. . . In contrast with the yield of 33.34 pounds of nitrogen per acre on 17 A, may be set the yield of 55.96 pounds on 20 A. The latter received the same amount and same kind of rye straw as 17 A but in addition to this it received also nitrate of soda at the rate of 320 pounds per acre. Thus a supply of readily available nitrogen runs the yield up even though no lime is applied. The effect of the supply of available nitrogen is likewise noted in 16 A. Here the nitrogen was furnished in the form of alfalfa hay chopped up and spread on the land before plowing, and since this furnished readily available nitrogen the yield was almost equal to the yield on 20 A which received one-third of its nitrogen in the form of nitrate of soda.

An interesting comparison may also be made between 8 A and 8 B. Plot 8 A received a very light application of nitrate of soda without lime, and yielded 23.77 pounds of nitrogen per acre in the crop. Plot 8 B received the same nitrogen treatment and also ground limestone and yielded 53.6 pounds of nitrogen per acre; as much as 9 B which received the double portion of nitrate of soda.

The high yield on both 18 A and 18 B is easily explained as a result of the heavy application of cow manure, and in addition nitrate of soda at the rate of 320 pounds per acre. These plots received nitrogen at the rate of 222.5 pounds per acre, much of it in a readily available form, while 17 A and 17 B received only 92.1 pounds, all of which was in a slowly available form. In spite of this fact 17 B, thanks to the influence of the lime, yielded almost as much total nitrogen as 18 B. The same point is brought out in the yields of nitrogen from plots 19 A and 19 B. Plot 19 A, to which no nitrogen or lime was applied, yielded nitrogen at the rate of 20.7 pounds per acre in the crop. Plot 19 B likewise received no nitrogen but it received the treatment of ground limestone and yielded

nitrogen at the rate of 32.1 pounds per acre in the crop, an increase over 19 A of more than 50 per cent.

Certainly these are not new facts, but, on the other hand, the figures do lay new emphasis upon truths which though known, have hitherto been too little regarded by the practical farmer, namely, that an abundance of phosphoric acid and potash cannot give a fair crop when nitrogen is the limiting factor and that even though there is an abundant supply of nitrogen along with the minerals, it cannot give a maximum crop if it exists in inert materials, that is, if it is not readily available during the growing period of the plant.

The average yield per acre of nitrogen for all unlimed plots was 38.06 pounds and for all limed plots 52.8 pounds.

It may well be pointed out again that the use of lime in the way indicated above carries with it the obligation of maintaining the supply of organic matter in the soil. Otherwise, a time will come when there will be little or no response to applications of lime.

#### PERCENTAGE OF NITROGEN RECOVERED

The last column in the table indicates the percentage of nitrogen recovered. This is obtained by subtracting the amount of nitrogen recovered from the check plots (the average of plots 4 and 19, these having received minerals but no nitrogen) from the total nitrogen recovered from any nitrogen treated plot. The amount of nitrogen recovered from the check plots is supposed to represent the soil nitrogen which the plant used, as distinguished from the applied nitrogen. The difference represents that part of the applied nitrogen which the plant was able to utilize. Therefore, when the amount of nitrogen that was applied is known, it becomes an easy matter to calculate the percentage of nitrogen that was recovered in the crop. It may be noted, however, that the percentages thus obtained may not be an entirely true indication of the amount of applied nitrogen recovered, for the reason that the plants on the nitrogen treated plots are stimulated to a greater use of soil organic nitrogen than the plants on the check plots.

Careful examination of the recoveries for the two sections brings out a number of points of interest. For example the average recovery for the B section is 11 per cent higher than the average for the A section. Likewise the average for plots 9 to 15 inclusive—those plots that receive nitrogenous compounds in equivalent amounts—is, for the B section 44.82 per cent and for the A section 34.05 per cent, a difference in favor of the B section of nearly 11 per cent.

It is of especial interest to note the low recovery in both sections, where excessive amounts of nitrogen were used, as for example plots 5 and 6 and 18 and 20. In these cases the recoveries were low both with and without lime, indicating a large loss of the applied nitrogen. Certain



of these plots, as for example 18 A and 18 B, gave rather heavy yields of grain, as compared with other plots, at least, but the cost of the treatment is too great for the returns secured. The cost of the nitrogen alone that was applied to these plots would not be less than \$20.00, perhaps nearer \$30.00, under normal conditions. Certainly such heavy applications are neither profitable nor economical. They are good illustrations of the law of diminishing returns. Evidently on these plots there was so much

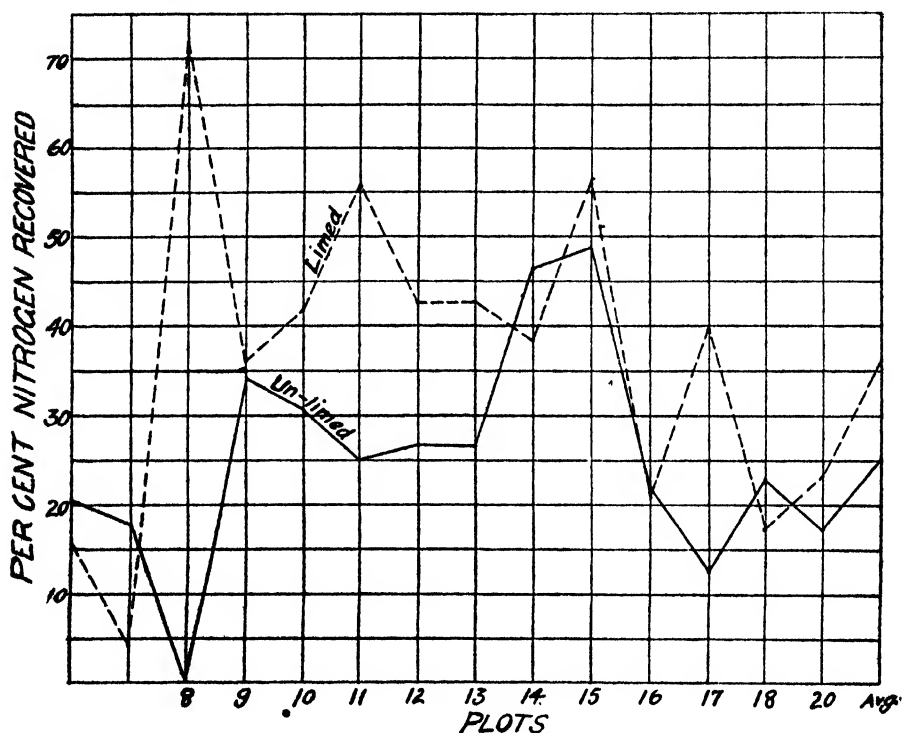


Fig. 3.—Percentage of nitrogen recovered from limed and unlimed plots: corn 1913.

available nitrogen applied that the lime did not have much influence one way or the other. In the case of plots 18 B and 20 B the possible influence of the basic effect of the soda of the nitrate of soda should not be overlooked. The heavy application of organic matter undoubtedly aided in improving the physical condition of the soil and in maintaining its supply of nitrogen, but such organic matter should be obtained in a more economical way.

The  $24\frac{1}{2}$  pounds of nitrogen per acre applied to plot 8 A does not seem to have been enough to increase the yield beyond the yield of the check plot, hence there was no recovery for this plot. The acid condition of the soil evidently resulted in a poor start for the corn and consequently it did not use, to good advantage, the small amount of available nitrogen that was at its disposal.

Plot 8 B, on the other hand, shows a recovery of nearly 72 per cent of the applied nitrogen. The correcting of the acidity and the improvement of the physical condition of the soil made it possible for the plant to utilize nearly three-fourths of the nitrogen that was applied.

A recovery of nearly 56 per cent of the applied nitrogen from plot 11 B, where lime was used in connection with ammonium sulfate, as against a recovery of 25 per cent on 11 A, which received the same treatment less the lime, indicates very clearly the importance of considering the reaction of the soil as a factor when ammonium sulfate is used.

Very much the same condition is noted with reference to plots 17 A and 17 B. The inert slowly available straw when used in connection with lime furnishes available nitrogen and shows a recovery on plot 17 B of 39.69 per cent as against 12.45 per cent on plot 17 A without lime.

It may be pointed out that the limed plots which receive calcium nitrate and calcium cyanamid show a higher recovery than similarly treated plots without lime.

The percentage of nitrogen recovered in the entire crop for the two sections is indicated by curves in figure 3.

#### SUMMARY

On a medium loam soil with a series of 20 twentieth-acre plots, arranged for a study of nitrogen availability, an application of ground limestone at the rate of 2 tons per acre, increased the yield of shelled corn by about ten bushels and of stover by 432 pounds per acre, as compared with the yield from a similar series of unlimed plots.

The influence of the lime on the yield from the plot which annually received its nitrogen in the form of ammonium sulphate, as compared with the yield from the similarly treated plot, unlimed, was especially marked.

The liming likewise resulted in greatly increased yields on certain of the plots which received their nitrogen in the form of rather slowly available organic materials, as, for example, wheat or rye straw. It also resulted in decided increases in the yields on plots which received minerals only, indicating that in the soil of these plots there was a considerable store of inert nitrogenous material which required only a favorable soil reaction to make it available.

Unlimed plots which received an extra heavy application of manure, or manure and nitrate of soda, gave yields fairly approaching or even surpassing the yields given by plots which received similar nitrogenous treatment and lime. That is, the manure or the basic materials in the manure and nitrate of soda apparently decreased the need for lime.

The average percentage of nitrogen in the grain and stover from the limed plots was slightly greater than the average in the grain and stover from the unlimed plots.

The average recovery of nitrogen from the limed plots was 36.2 per cent and the average from the unlimed plots was 25 per cent.

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# A RAPID METHOD FOR THE ESTIMATION OF CALCIUM OXIDE IN PEAT SOILS<sup>1</sup>

By

ROSS AIKEN GORTNER, *Associate Soil Chemist, Minnesota Agricultural Experiment Station, St. Paul, Minnesota*

It has recently been necessary to make a large number of analyses of peat soils at this experiment station, these analyses consisting of the estimation of nitrogen, volatile matter, residue insoluble in aqua regia, phosphoric acid and lime.

The ordinary method for estimation of calcium oxide consisting of the double precipitation of the iron and the aluminum hydroxides followed by a double precipitation of the calcium oxalate, consumed so much time that it was thought advisable to see if as accurate results could not be obtained by a shorter method, i. e. the precipitation of the calcium oxalate in the presence of the iron and aluminum hydroxides and subsequent titration with potassium permanganate.

Five grams of peat were incinerated in quartz dishes, the ash digested with aqua regia and then evaporated to dryness to dehydrate the silica, the residue taken up with dilute acid and filtered into a 500-c.c. flask. The filtrate was made to volume and constituted our Solution "A". This is the method followed by the Bremen Peat Experiment Station.<sup>2</sup>

The methods used are here given in detail. Method 2 is essentially the method for calcium as given by Washington.<sup>3</sup>

## METHOD 1

To 100 c.c. of Solution "A" enough ammonia is added to make the liquid smell strongly of it and to precipitate the iron and aluminum. The liquid is brought to a boil, preferably on a hot plate, and while boiling, 10 c.c. of a saturated solution of ammonium oxalate is added. By this procedure the calcium oxalate is precipitated over the surface of the iron hydroxide, making the latter more or less granular and greatly aiding filtration and washing. The boiling is cautiously continued for a couple of minutes and then the solution is allowed to cool. After at least 3 hours, (preferably over night) the solution is filtered through a 9-cm. filter (if

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<sup>1</sup> Received for publication May 8, 1916.

<sup>2</sup> König, S. *Untersuchung Landwirtschaftlich und gewerblich wichtiger Stoffe*. 9 ed., Paul Parey, p. 118-119. Berlin, 1911.

<sup>3</sup> Washington. *The Chemical Analysis of Rocks*, John Wiley & Sons, New York, 200 p., 1910.

there is only a small precipitate use a 7-cm. filter) and well washed with warm water. (A convenient test for the removal of the excess of oxalate is a solution of sulphuric acid containing 2 or 3 drops of standard permanganate solution. If 5 c.c. of the washings reduces the permanganate, it is shown that all of the ammonium oxalate has not been removed.)

When the precipitate is completely washed the beaker in which the precipitation was made is placed under the funnel and a hole punched in the filter paper. The precipitate is washed into the beaker with a stream of warm water and then the filter is well washed with a hot 1.5 sulphuric acid solution. Ten c.c. of concentrated sulphuric acid is added to the washings in the beaker and the solution brought to nearly a boil, when the oxalate is titrated with a solution of potassium permanganate each cubic centimeter of which is equivalent to 0.0010 gm. CaO. The number of cubic centimeters of permanganate used, divided by 10 gives the percentage of CaO.

#### METHOD 2 (Washington's Method)

To 100 c.c. of Solution "A," add enough ammonia to precipitate iron and aluminum hydroxides, boil and filter through a 9-cm. filter. Wash two or three times with hot water. Remove the filter paper and contents to the original beaker, and add 2 or 3 c.c. concentrated hydrochloric acid, break up the filter paper with a glass rod and add about 50 to 75 c.c. water, and a slight excess of ammonia. Boil, and filter, catching the filtrate in the same beaker as before. Wash the iron precipitate with hot water five or six times and then discard it. The filtrates are brought to nearly a boil, 10 c.c. of a saturated solution of ammonium oxalate added, the mixture allowed to cool, and 5 c.c. concentrated ammonia added.

After standing over night the mixture is filtered in a 7-cm. filter, being washed with *warm* water. The precipitate is dissolved on the filter with 1:5 nitric acid, and the solution received in the beaker in which the original oxalate precipitation was made. The filter is well washed with hot water and then dilute ammonia is poured through the filter paper to remove all traces of acid, the ammonia solution being caught in the original beaker containing the calcium. The contents of the beaker are heated to nearly boiling, made strongly alkaline with ammonia and 2 or 3 drops of ammonium oxalate solution added. After standing for at least 3 hours, the precipitated calcium oxalate is filtered through the same filter paper used above; washed with *warm* water, a hole punched in the filter and the precipitate washed through into the original beaker with 1:5 sulphuric acid. The filter is thoroughly washed, 10 c.c. of concentrated sulphuric acid is added to the contents of the beaker, and the solution brought to nearly boiling and titrated with permanganate as in Method 1.

Some of the results are shown in Table I. We have analyzed a large number of peat soils in this laboratory by both methods and have as yet found no peat soil in which the calcium could not be accurately estimated by the proposed modification. It may be, however, that such soils exist inasmuch as we have not had occasion to analyze peats containing less than 70 per cent of volatile matter.

TABLE I

COMPARATIVE CALCIUM OXIDE DETERMINATIONS BY THE PROPOSED NEW METHOD (METHOD 1) AND THE STANDARD METHOD (METHOD 2), USING PEAT SOILS

Soil No.		% Volatile	% Ash	% Insol.	% CaO Method 1		% CaO Method 2	
					I	II	I	II
A	(Analyst A)	78.68	21.32	10.26	4.63	....	4.59	4.65
B	(Analyst A)	90.59	9.41	6.12	0.63	....	0.62	0.64
B	(Analyst B)	.....	.....	.....	0.65	...	0.60	....
C	(Analyst A)	86.10	13.90	11.37	1.22	...	1.20	1.21
C	(Analyst C)	....	..	....	1.24	1.24	1.25	....
C	(Analyst D)	.....	.....	..	1.25	1.24	1.14	1.14
D	(Analyst E)	90.03	9.97	4.96	2.21	2.21	2.31	2.28
E	(Analyst E)	88.75	11.25	7.23	1.53	1.54	1.53	1.55
F	(Analyst E)	88.77	11.23	7.03	1.56	1.60	1.53	1.59
G	(Analyst E)	91.36	8.64	6.71	0.54	0.56	0.53	0.55
H	(Analyst E)	91.31	8.79	6.95	0.48	0.43	0.48	0.48
I	(Analyst E)	86.47	13.53	8.99	1.52	1.63	1.52	1.57
J	(Analyst E)	92.22	7.78	5.58	2.73	3.00	2.87	2.93
K	(Analyst E)	86.98	13.02	6.86	3.22	3.12	3.16	3.20

TABLE II

COMPARATIVE CALCIUM OXIDE DETERMINATIONS BY THE PROPOSED NEW METHOD (METHOD 1) AND THE STANDARD METHOD (METHOD 2), USING A MINERAL SOIL

	Method 1		Method 2	
	I	II	I	II
Total CaO				
(Carbonate fusion) .....	4.27	4.29	3.60	3.61
Acid soluble CaO				
12 hr. 1.115 Sp. Gr. HCl .....	2.68	2.71	2.59	2.39
5 days 1.115 Sp. Gr. HCl .....	3.27	3.28	2.84	2.84
5 Min. 5% HCl .....	2.66	2.72	2.51	2.51

In an attempt to apply the method to mineral soils it was found that results which were much too high were invariably obtained, even in acid extracts. Some of these results are shown in Table II. Just what factors cause the high results for mineral soils has not been determined.

## SUMMARY

Calcium can be accurately determined in the acid extract of the ash of those peat soils which contain a high percentage of volatile matter by a single precipitation with ammonia and ammonium oxalate *in the presence of the iron and aluminum hydroxides* and subsequent titration of the precipitate with permanganate.

The method is not applicable to mineral soils.

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### PROTEIN DECOMPOSITION IN SOILS<sup>1</sup>

BY ELBERT C. LATHROP, *Biochemist in Soil Fertility Investigations, U. S. Department of Agriculture*

#### INTRODUCTION

From the standpoint of soil fertility the nitrogenous portion of the soil organic matter is of undoubted importance. Evidence, both direct and indirect, has been obtained which indicates that the larger portion of the nitrogenous matter of soils either is composed of proteins themselves or has been derived from proteins.

A number of investigators, working with soils from widely separated localities and of totally different origin and occupation, have shown that when the soils are treated by boiling with strong mineral acids the greater portion of the nitrogenous material goes into solution, whereas, before this treatment, it had been practically insoluble in the cold acids. It has been demonstrated, furthermore, that the acid solutions from the soils so treated gave, on analysis for the various forms of nitrogen, results very similar to those obtained by acid hydrolysis of proteins themselves. By means of isolation methods guanine (23), hypoxanthine, xanthine, arginine, and cytosine (46), decomposition products of nucleoproteins, have been obtained by partial hydrolysis of a soil with steam heat, though not found in the soil before heating. In addition, leucine and isoleucine have been obtained from Michigan peats (43) after hydrolyzing by boiling with strong acid. Such, in brief, is the indirect evidence for the occurrence of protein substances in soils.

The direct evidence of the protein nature of some of the nitrogenous portion of soil organic matter has been obtained almost entirely by Schreiner and his colleagues, Shorey, Lathrop and Walters, in their investigations on the composition of the organic matter of soils (48, 49, 45, 46, 53, 54, 55, 56, 60). These investigators have so far succeeded in isolating from soils the following nitrogenous compounds related to the proteins: proteoses and peptones; nucleic acids; the diamino acids, arginine, histidine and lysine; the pyrimidine base, cytosine; the purine bases, xanthine, hypixanthine and adenine; the base, choline, a decomposition product of phosphoproteins; and finally, creatinine, trimethylamine, tetra-carbonimid and picoline carboxylic acid, most, if not all, possible secondary protein decomposition products. Very recently Potter and Snyder

<sup>1</sup> Received for publication May 13, 1916.



(41, 42), by the use of the Kober method (22), have been able to show that in some soils, at least, the amounts of free amino acids and peptides are very low.

These compounds, isolated from soils, together with many other decomposition products of proteins, have been investigated in regard to their action on plant growth (14, 47, 50), and it has been found that a large number of them are of direct value in plant nutrition, while others are toxic. A number of these can not only be used by the plants as a source of their nitrogen requirements but it has been shown that in water culture solutions, in the presence of nitrates, they are utilized by plants quite as readily as the nitrates themselves, thus indicating that these compounds may efficiently act as nitrate sparsers.

Considered also from the standpoint of the energy expended by the plant in its metabolism, these compounds may play a very important part. Less energy, for example, is probably required in the synthesis of the plant proteins from compounds such as the amino acids or purine bases, which contain not only assimilable nitrogen, but carbon, hydrogen and oxygen as well, than in the synthesis of these same proteins from nitrate or ammonium salts alone, where the simpler components of the protein must in all probability be first synthesized and then these combined to complete the process. In the light of such a view it becomes of interest to observe that in the process of the decay of proteins in the soil they are split up first into the simpler compounds, such as peptides and amino acids, and that the greater portion of the ammonia is derived from these. These compounds are therefore presented to the plant at an earlier period in the decay of the protein than if the nitrogen compounds, in order to be utilized by the plant, had first to be changed into ammonia and then into nitrates.

Since in agricultural practice, protein material, of plant and of animal origin, is continually being added to the soil, it becomes necessary, from the practical and from the theoretical viewpoint as well, to obtain as accurate a picture as is possible of the changes which take place in proteins after they are introduced into the soil. This is especially important in connection with the interpretation of the action and availability of commercial organic fertilizers, barn-yard manure and green manures.

This investigation was therefore undertaken for the purpose of studying the changes taking place in protein material when added to an agricultural soil. Since ammonia formation is but one step in the process it becomes of interest to know from what portion of the protein molecule this ammonia is derived; to determine for how long a time the protein itself or the primary components of the protein can persist in the soil, and finally to get some insight into the nature of the protein compounds formed by the action of the microorganisms in their life processes.

TABLE I  
COMPOSITION OF THE PROTEINS OF HORSE BLOOD AND CATTLE BLOOD  
Results expressed in per cent

Amino acid	Globin of the oxy-hemoglobin of horse blood	Serum albumin of horse blood	Serum globin of horse blood	Nonpurified fibrin of cattle blood
Glycocoll .....	10.00	20.00	23.52	22.00
Alanine .....	14.19	22.68	22.22	23.60
Leucine .....	129.04	20.00	18.70	15.00
Phenylalanine ...	14.24	23.08	23.84	22.50
Proline .....	12.34	21.04	22.76	23.60
Glutamic acid ....	11.73	21.52	22.20	22.50
Aspartic acid ....	14.43	23.12	22.54	22.00
Cystine .....	10.31	22.53	20.67	....
Serine .....	10.56	20.60	....	20.80
Oxyproline .....	11.04	...	...	...
Tyrosine .....	11.33	22.10	...	23.50
Valine .....	...	...	....	21.00
Lysine .....	14.28	....	....	....
Arginine .....	15.42	...	...	...
Histidine .....	10.96	...	...	...
Tryptophane .....	1+	2+	...	...
Ammonia .....	11.07	21.01	21.75	...
Cystein .....	....	4+	...	...

<sup>1</sup> E. Abderhalden (2). <sup>2</sup> E. Abderhalden (4). <sup>3</sup> E. Abderhalden (3). <sup>4</sup> G. Emlen (9).

<sup>5</sup> K. A. H. Morner (37). <sup>6</sup> W. Hausmann (11). <sup>7</sup> W. Hausmann (12).

<sup>8</sup> E. Abderhalden (5). <sup>9</sup> E. Abderhalden (6)

## EXPERIMENTAL

### *Dried Blood*

Dried blood which was chosen for this investigation, in addition to its suitability as a material high in protein matter, is a high grade nitrogenous fertilizer; that is, according to all tests it is of a high degree of availability for plant use and is used as a fertilizer in many sections of the United States and elsewhere, as such, and in mixed fertilizers. It is composed almost entirely of various animal proteins and the commercial product is of fairly constant composition. Abderhalden (1) reports figures on the composition of the blood of cattle, sheep, pigs, horses and goats, which show that a mixture of the blood of these animals should contain about 200 parts of solid matter for 1000 parts of blood. These solids consist of about 54 per cent hemoglobin and about 32 per cent albumin, or approximately 86 per cent proteins, exclusive of any nucleoproteins or nucleic acids which also are undoubtedly present. The products of the acid hydrolysis of the proteins of horse blood, globin of the hemoglobin, serum albumin and serum globin, and the non-purified fibrin of the blood of cattle, have been estimated in part by several investigators and the results so obtained are presented in Table I. The method used for the separation and estimation of the various amino acids is the esterification method proposed by E. Fischer, which is not strictly quantitative, involving losses

in the amounts of many of the amino acids, so that the figures obtained represent less than the actual amounts of the various hydrolysis products of these proteins.

The dried blood used in this investigation was purchased in the open market and contained 13.92 per cent of total nitrogen. Two 3-gm. samples of the dried blood were hydrolyzed by boiling with 60 c.c. of hydrochloric acid, sp. gr. 1.115 for 18 hours, after which time a positive biuret test could no longer be obtained, showing complete hydrolysis. The various forms of nitrogen in the hydrochloric extract were then estimated according to the nitrogen partition method proposed by Van Slyke (57), and the results so obtained are presented in Table II. Cystine nitrogen was not determined for the reason that it was thought that this determination would be of little value when studying the decomposition products of dried blood in soils; consequently any cystine nitrogen present is included with the nitrogen estimated as arginine, histidine and lysine.

### *The Soil Used*

The soil was a Norfolk fine sandy loam taken from a cantaloup field near Raleigh, N. C. The soil was in a high state of cultivation and had received both mineral fertilizers and stable manure. It was found to contain 0.0301 per cent total nitrogen. The soil was passed through a 40 mesh sieve and dried *in vacuo*.

Forty parts of soil were mixed with about three parts of dried blood by sieving the two together repeatedly until samples taken from different parts of the mixture gave duplicate analyses for total nitrogen. The total nitrogen in the soil thus prepared was determined by the Kjeldahl-Gunning-Arnold method and was found to be 0.8945 per cent. The ammonia in the soil was determined by the vacuum distillation method recommended by the author (24) for the determination of ammonia in processed fertilizers and was found to be 0.0005 per cent. It should be stated that all analytical figures reported in this investigation are calculated on the oven-dried basis.

TABLE II

THE FORMS OF NITROGEN IN DRIED BLOOD AND IN THE EXPERIMENTAL SOIL  
Results expressed in per cent of hydrolyzable nitrogen

Form of Nitrogen	Dried Blood	Experimental Soil
Amide nitrogen .....	6.854	7.008
Melanin nitrogen .....	2.600	4.767
Arginine nitrogen .....	7.517	7.601
Histidine nitrogen .....	12.523	12.366
Lysine nitrogen .....	11.517	10.093
Monoamino acid nitrogen .....	57.057	58.220
Non-amino nitrogen .....	1.479	0.312
Total .....	99.547	100.367

The soil was made up of 10 per cent moisture content, was kept in a 1-gallon stone-ware jar covered with perforated wrapping paper to exclude dust, and the decomposition was allowed to proceed at the temperature of the laboratory.

During the first 18 days the soil was kept at a constant moisture content of 10 per cent and was mixed several times by hand during that period to promote aeration. Later on, however, the soil was made up to 10 per cent moisture content every 5 to 8 days and on two occasions was allowed to dry out. At each addition of water to the soil it was dumped out of the jar and thoroughly mixed to promote aeration. The total length of the experiment was 240 days, during which time samples of the experimental soil were taken at the following intervals after it had been prepared: (1) 18 days, (2) 44 days, (3) 86 days, (4) 148 days, and (5) 240 days.

At the end of each period the soil was sampled, after a thorough mixing, by means of a brass tube which took a core of the soil from top to bottom. About eight borings were made at each sampling from different parts of the jar so that a fairly representative fraction of the soil was obtained. These different borings, amounting to about 300 gm. of moist soil, were then mixed well and placed in a small mason jar. All of the weighings for the analytical work were immediately made. In order to make sure that the sample in the mason jar was uniform, total nitrogen determinations were made on portions taken from the top and the bottom of the jar.

The dry mixture of soil and dried blood which was not used in preparing the experimental soil was placed in a glass stoppered bottle; total nitrogen and ammonia determinations made on samples of this taken from time to time showed that under such dry conditions no decomposition was taking place.

The amount of moisture in the various samples was determined by drying them in an oven for 2 hours at 103° C. Total nitrogen and ammonia determinations were made on samples of the experimental soil at the end of each period according to the methods already mentioned. Nitrates were not determined. One-hundred-gram samples of the soil at each sampling were subjected to hydrolysis by boiling with 200 c.c. of hydrochloric acid, sp. gr. 1.115, for 48 hours. The acid solution was filtered from the soil by suction and the soil was washed with boiling water until the washings became neutral in reaction. The combined acid filtrate and washings were concentrated at 40° C. under 10 mm. pressure to a thick syrup in order to get rid of most of the hydrochloric acid. The residue was taken up in hot water, the aqueous solution was filtered into a 250 c.c. volumetric flask and the filter thoroughly washed with hot water. After cooling, the solution was made up to the mark and total nitrogen

determinations were made on two 25-c.c. portions. The remainder of the respective solutions were subjected to the determination of the different forms of nitrogen, the details of the method as outlined by Van Slyke (57) being followed, excepting that the determination of cystine nitrogen was omitted.

### *The Analytical Results*

By the use of the methods outlined the nitrogen was separated into the following: (1) total nitrogen in the soil, (2) total nitrogen in hydrochloric acid solution, (3) ammonia nitrogen in the soil, (4) ammonia nitrogen in the hydrochloric acid solution, (5) melanin nitrogen, (6) nitrogen precipitated by phosphotungstic acid, reported as arginine, histidine and lysine nitrogen, (7) nitrogen in the filtrate from the phosphotungstic acid precipitate, reported as monoamino acid nitrogen and non-amino nitrogen.

By subtracting the amount of ammonia nitrogen found in the soil (3) from the amount of ammonia nitrogen found in the hydrochloric acid extract (4) the amount of nitrogen in the soil in the form of the amide group in proteins or as acid amides may be obtained; this is reported as amide nitrogen. The amount of nitrogen in the soil in the form of proteins or protein decomposition products, with the exception of ammonia nitrogen, may be obtained by subtracting the amount of ammonia nitrogen in the soil (3) from the amount of total nitrogen in hydrochloric acid solution (2); this is reported as "hydrolyzable" nitrogen. The amount of nitrogen in all of the various fractions was determined by the Kjeldahl method, which does not include nitrate nitrogen unless large amounts of reducing substances are present. Such may be the case, however, with some of the Kjeldahl analyses and any nitrate nitrogen, therefore, included in a Kjeldahl determination would be reported as non-amino nitrogen.

In a recent article Van Slyke (58) states that the method which he has proposed for the partition of nitrogen was designed for use only with proteins not accompanied by other classes of substances, particularly nitrogenous substances, which would obviously falsify the interpretation of the results unless the behavior of the non-protein substances is so accurately known that corrections might be made. It should be clearly understood and constantly borne in mind that after the decomposition in the soil for any length of time of such complex organic compounds as those contained in dried blood, undoubtedly compounds other than proteins or the primary products of protein decomposition must make their appearance. Just what these compounds may be we can but conjecture at the present time, so that the results of the Van Slyke method when applied to the partition of the forms of nitrogen in soils, while reported as arginine nitrogen, histidine nitrogen, etc., can be considered as being only ap-

proximations for the amounts of these various forms of nitrogen actually present in the soil as proteins or as the primary products of protein decomposition. It should be clearly emphasized, however, that the method is of decided value, even under limiting circumstances, in attacking such a problem as the one at hand, since by the use of such a method it is possible to divide the nitrogenous compounds present in the soil into a number of classes which react towards the various reagents involved in the analytical procedure as though they were arginine nitrogen, histidine nitrogen, etc. The very fact that a given nitrogenous compound will, towards a given chemical reagent or a series of them, react like arginine, histidine, etc., establishes a chemical and possibly a biochemical relationship.

In regard to the nitrogen reported in this investigation as amide nitrogen it might be stated that it is difficult to conceive in the present state of our knowledge of any soil compounds other than the amide group of the various proteins, or the acid amides themselves, which would resist heating *in vacuo* with calcium hydroxide and subsequently split off ammonia on heating with hydrochloric acid.

The melanins are at present undefined and no significance can be attached to the figures obtained.

The nitrogen reported as monoamino nitrogen includes all nitrogenous compounds not precipitated by calcium hydroxide or not volatile in its presence *in vacuo*, not precipitated by phosphotungstic acid and containing a free amino group which will react with nitrous acid to produce free nitrogen.

The greatest inaccuracies occur in the diamino acid fraction and these are distributed between arginine, histidine and lysine nitrogen. This group includes all nitrogenous compounds which are precipitated by phosphotungstic acid, excepting the ammonia and melanin nitrogen which have been previously removed.

The nitrogen reported as non-amino nitrogen includes all nitrogenous compounds not accounted for in the above and may include a small amount of nitrogen present in the soil in the form of nitrates.

The results obtained by the methods outlined are presented in Tables IV and V.

### *Hydrolysis*

The amount of dried blood added to the Norfolk fine sandy loam is far in excess of the amount ever added in good agricultural practice. However, this amount was found by experiment to be necessary in order to obtain accurate analytical results; furthermore, it seemed desirable to add enough dried blood protein to the soil to render the small amount of soil protein negligible, so that only the fertilizer nitrogen would be under observation. By reference to Table II, in which the results of the Van

Slyke method as applied to the mixture of blood and soil are reported, it will be observed that the figures obtained for the various forms of nitrogen correspond very closely to those obtained from the dried blood alone, except the figures for melanin and non-amino nitrogen, but the reason for this is not altogether clear.

Under natural conditions the changing of organic nitrogen into ammonia is the work of microorganisms in the soil. Müntz and Coudon (38), studying the ammonification in sterilized and unsterilized soil, showed that during two and one-half years there was no ammonia formation in the sterilized soil, while the unsterilized soil in 67 days produced from 41 to 110 mg. of ammonia per 100 gm. of soil. Ammonia formation during the bacterial or mold decomposition of protein materials is an evidence of chemical changes in the protein molecule and the total amount of ammonia formed during the entire decomposition process may in a general way, perhaps, be considered an index of the extent of these changes. The interest in the present investigation centers in establishing the actual chemical origin of this ammonia, the portions of the protein molecule from which it is split by the soil organisms and thus elucidating the chemical changes involved in the disappearance of this type of organic matter from soils. It is obvious that for a full appreciation and understanding of these biochemical changes which occur during the decay of proteins in the soil, a knowledge of the molecular structure of the proteins and of the mechanism of microorganismal action is very essential.

The synthetic researches of Emil Fischer and his pupils, begun in 1901, on the structure of the protein molecule, prove the accuracy of Hofmeister's (13) view that the acid amide combination of the amino acids is the principal one in the protein molecule, according to the general structure:



The chemical nature of an albumin is apparently partly determined by the quantitative relationships of the different amino acids and partly by the arrangement of these amino acids in the protein molecule. Two points regarding the constitution of the protein molecule have been fairly conclusively established, which have a direct bearing on this study. A small portion of the total nitrogen of the protein molecule is liberated as ammonia on hydrolysis; this points to the presence of linkings in the form of acid amide ( $\text{—CO—NH}_2$ ) combinations. From a study of the amounts of ammonia formed by the hydrolysis of a large number of proteins by acids and the amounts of ammonia formed by heating these proteins with a solution of sodium hydroxide, Osborne, Leavenworth and Brautlecht (40) conclude that it is highly probable that the ammonia results from an amide union in the protein molecule. Van Slyke and Birchard (59) from a study of the action of certain proteins towards

nitrous acid, conclude that one of the two amino groups of lysine, the  $\omega$ -group, exists free in the protein molecule. This group represents within, at most, a fraction of a percentage of the protein nitrogen, the entire amount of free amino nitrogen determinable in the native proteins by the nitrous acid method. The  $\alpha$ -groups, which constitute the remaining and greater part of the free amino nitrogen found after complete hydrolysis, are in the intact protein molecule practically all condensed into peptide linkings. With primary albumoses, the first decomposition products of proteins, the relations are different; the free amino nitrogen in hetero- and protoalbumoses exceeds half of the lysine nitrogen by 3.00 and 4.80 per cent of the total nitrogen respectively, indicating that an appreciable portion of the  $\alpha$ -amino groups of other amino acids is uncovered even in primary digestion products.

Hydrolysis of the protein molecule by means of various chemical reagents and enzymes results in the introduction of water into the molecule at various places with the appearance of albumoses, peptones, polypeptides, amino acids and ammonia, the amounts and nature of the products depending on the nature of the protein and the specific reagents used. The final products of acid hydrolysis are the amino acids and ammonia, while with pepsin no amino acids are said to be formed, the splitting resulting in the formation of albumoses, peptones, peptides and ammonia. Trypsin differs from pepsin in that, although it cannot attack all proteins, requiring in some instances the action of pepsin first, it splits the molecule more deeply, with the formation of amino acids, together with many of the products formed by peptic digestion.

In regard to the decomposition of proteins by microorganisms, numerous investigations have been made and the following general conclusions may be drawn from them. There is little reason to suppose that the action of microorganisms is other than that of the enzymes which they produce. Kruse states that bacterial proteolytic enzymes resemble both pepsin and trypsin in the nature of their action but are different from either. The degradation of proteins by microorganisms proceeds along the same general lines as that produced by proteolytic enzymes and acids but the process does not stop with hydrolytic cleavage, a deeper change taking place with the formation of large amounts of ammonia and carbon dioxide, together with amines, fatty acids, alcohols, aldehydes, hydrogen sulfide, methane, phenol, skatol, indol, etc.

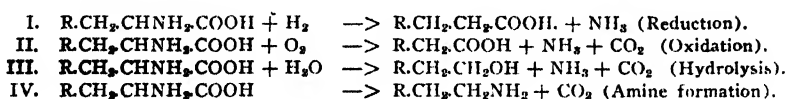
#### *Ammonia Production*

Two sorts of splitting by which ammonia is formed deserve consideration: first, the production of ammonia by direct hydrolysis of the proteins, with the consequent destruction of the amide group, ( $-\text{CO}-\text{NH}_2$ ) contained in the proteins; second, the formation of ammonia from other portions of the protein molecule. For instance, if ammonia were formed



by the splitting off of only the amide group from the proteins of the dried blood, then, the total amount of ammonia produced during the entire decomposition would amount to about 7.0 per cent of the total nitrogen of the fertilizer. However, as may be seen from the results presented in Table III, the total amount of ammonia nitrogen produced during the 240-day period in the soil represents about 79.0 per cent of the total nitrogen originally present in the dried blood. It is evident, therefore, that ammonia has been formed from other fractions of the protein molecule besides that containing the amide linking.

In regard to the action of microorganisms on amino acids it may be stated that the chemical changes involved depend largely upon the character of the organisms, the condition of growth especially with regard to the presence or absence of oxygen, and the available sources of nutrient other than amino acids. In general, it may be said that anaerobic bacteria are prone to reduce  $\alpha$ -amino acids with the formation of fatty acids and the liberation of ammonia, (equation I). Aerobic bacteria more frequently oxidize the  $\alpha$ -amino acids to a fatty acid containing one less carbon atom, carbon dioxide and ammonia being set free (equation II). Yeasts have been shown by Ehrlich to convert amino acids into alcohols, carbon dioxide and ammonia (equation III), the net result of this reaction indicating neither oxidation or reduction but simple hydrolysis with carbon dioxide liberation. Another type of reaction (equation IV) very commonly brought about by bacteria involves the liberation from amino acids of carbon dioxide but not ammonia; it is in this manner that amines may be formed. The type reactions involved in these various changes may be represented as follows (8):



A combination of a number of these reactions may be effected by a single organism and different results may often be obtained using the same organism under varying conditions.

The investigations concerned with the process of ammonification in the soil cover a large number of years and a résumé of this work is not deemed essential. However, among the investigations more recently conducted may be mentioned those by Löhnis (33, 34, 35), J. G. Lipman and his co-workers (27, 28, 29, 30, 31), C. B. Lipman and P. S. Burgess (26), P. E. Brown (7), W. P. Kelly (20), W. G. Sackett (44) and H. C. McLean and G. W. Wilson (36). From the results obtained by these investigators and others it is apparent that there are many factors which are involved in the process of ammonification of organic nitrogen. Some of these factors are: soil moisture, aeration of the soil, the mineral salts present, the physical and chemical nature of the nitrogenous matter, the

amount of organic matter present and the depth of the layer through which this is distributed, and the type and number of the organisms at work in the soil.

Assuming that ammonification of protein material in soils must precede nitrification and denitrification and that all loss of nitrogen in this investigation is due to ammonia evaporation, nitrification or denitrification, and that free nitrogen is not split off from compounds other than nitrates or nitrites, then it is possible to arrive at the amount of ammonia formation in the soil during each period of time. It should be stated that this is ammonia formed exclusive of ammonia assimilated, there being no way in which ammonia assimilation could be accurately determined in this experiment.

This ammonia formation may be calculated from the following equations:

Total  $N-NH_3$  nitrogen in the original soil = A.

Total  $N-NH_3$  nitrogen in soil at end of each period = B.

Then  $A-B=X$ , or ammonia formation during the period.

X

— = per cent of nitrogen changed to ammonia during the period.

A

TABLE III

PER CENT OF TOTAL NITROGEN IN THE SOIL AMMONIFIED AT THE END OF EACH PERIOD OF SAMPLING

Time from the beginning of the experiment	Per cent of total nitrogen
18 days .....	18.72
44 days .....	54.03
86 days .....	72.66
148 days .....	78.13
240 days .....	78.92

Table III, in which are presented the results obtained by the use of the above formula, shows that about 79 per cent of the nitrogen of the dried blood was converted to ammonia in 240 days. At the end of 86 days, less than half the total length of the experiment, about 73 per cent of the nitrogen of the dried blood had been changed into ammonia, showing that not only was the amount of ammonia formed during the remaining 154 days very small but that the rate of ammonification of the nitrogenous matter of the soil was greatly reduced, being about 10 per cent of the rate during the first period of 18 days.

#### *The Results of the Van Slyke Analysis*

By comparing the results obtained by the Van Slyke analysis of each soil sample during the experiment with the results obtained on the original soil the amounts of gain or loss in the eight different forms of nitrogen can be arrived at. It is thus possible to determine how rapidly any

particular form of nitrogen compound disappeared from the soil in the course of the decomposition and, further, to determine the relative amounts of nitrogen in these fractions in respect to the total amount of nitrogen present in the soil at the end of any period. When an increase in any particular form of nitrogen over the amount present in the soil during the previous period is observed it is not possible in all cases to state the compound in which this nitrogen existed, but when a certain form of nitrogen shows a loss during a period it is an absolute indication that that particular kind of nitrogen was disappearing or had disappeared from the soil, although the rate could not be determined. The results obtained by these analyses are presented in Table IV, in which the amounts of nitrogen in the various fractions are reported in per cent of the hydrolyzable nitrogen of the original soil. The results were all obtained by direct analysis, except in the fifth period when the melanin nitrogen was obtained by difference.

TABLE IV  
THE FORMS OF NITROGEN IN THE SOIL AT THE END OF EACH PERIOD  
Hydrolyzable nitrogen in the original soil = 100

Forms of nitrogen	Original soil	Time in days from the beginning of the experiment				
		18	44	86	148	240
Amide nitrogen .....	7.008	7.515	6.025	5.429	3.454	3.222
Melanin nitrogen .....	4.767	5.080	4.374	2.276	1.391	1.698
Arginine nitrogen .....	7.601	5.162	3.041	1.857	1.342	1.395
Histidine nitrogen .....	12.366	12.975	5.547	2.912	2.382	2.010
Lysine Nitrogen .....	10.093	7.610	1.110	0.429	0.528	0.972
Monoamino acid nitrogen .....	58.220	40.493	18.612	8.970	7.938	7.187
Non-amino nitrogen .....	0.312	1.120	1.675	2.191	0.738	0.297
Hydrolyzable nitrogen .....	100.000	79.660	40.598	24.070	17.740	16.741

The figures presented in Table V represent the relative amounts of the various forms of nitrogen in percentages of the hydrolyzable nitrogen of the soil present at the end of each period. From this table the fluctuating composition of the hydrolyzable nitrogen of the soil may be followed and the final composition of the hydrolyzable nitrogenous matter of the soil may be established.

TABLE V  
THE FORMS OF NITROGEN IN THE SOIL AT THE END OF EACH PERIOD  
Hydrolyzable nitrogen in the soil at the end of each sampling period = 100

Forms of nitrogen	Original soil	Time in days from the beginning of the experiment				
		18	44	86	148	240
Amide nitrogen .....	7.008	9.555	14.840	22.556	19.471	19.246
Melanin nitrogen .....	4.767	6.375	10.773	9.453	7.657	10.910
Arginine nitrogen .....	7.601	6.477	7.491	7.717	7.567	8.333
Histidine nitrogen .....	12.366	16.276	13.663	12.099	13.421	12.006
Lysine nitrogen .....	10.093	9.550	2.710	1.784	2.979	5.809
Monoamino acid nitrogen .....	58.220	50.812	45.847	37.264	44.745	42.922
Non-amino nitrogen .....	0.312	1.410	4.125	9.102	4.160	1.774
Hydrolyzable nitrogen .....	100.000	100.000	100.000	100.000	100.000	100.000

The figures presented in Table VI show the amounts of loss of nitrogen in each form in the soil at the end of each sampling period. The amount of loss is stated in percentages of the largest amount of any form of nitrogen in the soil at any time; for example, in the case of the amide nitrogen the amount is largest at the end of 18 days, and this figure is taken as 100. In this table the word "gain" indicates an increase in the amount of nitrogen over that present at the end of the preceding period.

TABLE VI  
THE PERCENTAGE LOSS OF THE VARIOUS FORMS OF NITROGEN IN THE SOIL AT THE END OF EACH SAMPLING PERIOD  
The largest amount of nitrogen in the soil = 100

Form of nitrogen	Time in days from the beginning of the experiment				
	18	44	86	148	240
Amide nitrogen .....	Gain	20	28	55	57
Arginine nitrogen .....	31	60	76	83	Gain
Histidine nitrogen ..	Gain	58	80	82	83
Lysin nitrogen ..	24	89	96	Gain	Gain
Monoamino acid nitrogen .....	31	67	84	86	89
Hydrolyzable nitrogen .....	20	59	76	82	83

### *Hydrolyzable Nitrogen*

During the 240-day decomposition of the dried blood in the soil a loss of 83 per cent of the total hydrolyzable nitrogen took place. At the end of 86 days the loss was 76 per cent, showing that during the latter and longer portion of the decomposition experiment the amount of hydrolyzable nitrogen which vanished from the soil was extremely small.

### *Monoamino Acid Nitrogen*

During the experiment the monoamino acid nitrogen diminished from 58 to 7 per cent, or a loss of 89 per cent of the total monoamino acids originally present in the proteins. At the end of 18 days, 31 per cent of this form of nitrogen had vanished, while during the same time only 20 per cent of the hydrolyzable nitrogen was lost. Since the monoamino acids contain more than half of the total hydrolyzable nitrogen, it appears that the relative loss from each would be about the same. The fact that there is a difference of about 11 per cent between the losses from these fractions leads to the supposition that nitrogen split off from the monoamino acids has been assimilated by the microorganisms in the formation of their protoplasm.

It may be stated in this connection that it has been found by the few investigations concerned with the chemical nature of the protoplasm of microorganisms that this protoplasm is composed to a greater or less extent of proteins depending somewhat upon the nature of the media upon which the organisms have developed. Regarding the general nature of the proteins of bacteria and mold protoplasm a number of investigations

have been conducted, but aside from the isolation of some protein-like substances and some nucleic acids from this sort of protoplasm, together with the isolation of some amino acids from the hydrolysis products of these substances, not much is actually known concerning the real chemical composition and structure. In regard to the nitrogen compounds which are present in the protoplasm of soil organisms, Omelianski and Sieber (39) report that the bodies of *Azotobacter chroococum* contain about 13 per cent of nitrogen, which, by analysis according to the Van Slyke method, they found to be distributed as follows: amide nitrogen 9.6, melanin nitrogen 3.5, arginine nitrogen 10.13, histidine nitrogen 1.64, lysine nitrogen 14.60, monoamino acid nitrogen 55.40, and non-amino nitrogen 4.86 per cent, respectively, of the total hydrolyzable nitrogen. The composition of the protein of other organisms would probably differ.

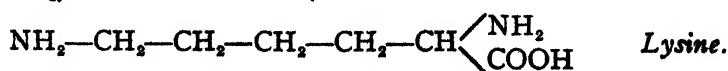
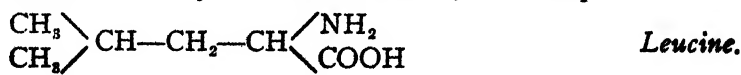
In Table V it will be observed that the proportion of the monoamino acids present in the soil at the various times of sampling fluctuates. The lowest figure is 37 per cent at the end of 86 days.

### *Lysine Nitrogen*

The analytical results show that lysine disappears from the soil quite rapidly. At the end of 44 days, 89 per cent of the lysine originally present in the proteins has been decomposed, and at the end of 86 days, 96 per cent. During the remaining and longer part of the decomposition period there is a continual gain in lysine nitrogen, indicating that synthetic processes are at work.

The gain in lysine nitrogen, after the original had practically vanished from the soil, is to be attributed to the action of the microorganisms in synthesizing some compound or compounds which give the analytical reactions for lysine. That this increase is due entirely to lysine cannot be stated, but lysine no doubt makes up a part of the gain observed.

It will be noted from Table VI that the two fractions which show the greatest amount of loss during the experiment are lysine nitrogen and monoamino acid nitrogen. It is not surprising that these two show the greatest loss when their chemical composition is considered. The monoamino acids are straight chain acids with the amino group in the alpha position to the carboxyl group. Lysine, a diamino acid, is also a straight chain acid containing two amino groups, one in the alpha position to the carboxyl group and one at the extreme end of the chain from the carboxyl group, or in the omega position. The relationship between lysine and the amino acids may be clearly shown by presenting the structural formulas for lysine and for leucine, for example:



However, it is observed that the lysine vanishes more quickly from the soil than the monoamino acids. This may be due to the fact that the  $\omega$ -amino group of the lysine exists free in the molecule of the native proteins which occur in the dried blood. Under such conditions this group is subject to deaminization by the action of the microorganisms before hydrolysis takes place, while in the case of the monoamino acids hydrolysis must precede deaminization since these acids are linked in the protein molecule in anhydride structure. Furthermore, if the omega group be split off from the lysine while it is still a constituent part of the protein molecule it is changed into an amino acid with but one amino group and would be determined analytically as monoamino acid nitrogen.

From Table V it will be observed that there are very marked fluctuations in the proportions of lysine nitrogen in the soil at the end of each period. The lowest amount occurs in the soil at the end of 86 days, which was the low point for monoamino acids. The final amount is about half that originally present in the dried blood.

#### *Histidine Nitrogen*

At the end of 18 days the histidine nitrogen showed a gain. Although the compounds which cause this increase cannot be arrived at, it is possible that they are, in part at least, the purine and pyrimidine bases, which by the analytical methods would be classed as histidine nitrogen. It is well known that the protoplasm of microorganisms is made up of considerable amounts of nucleoproteins and nucleic acid, which on hydrolysis would yield the purines and pyrimidines.

At the end of 44 days 60 per cent of the histidine nitrogen had disappeared; at the end of 86 days, 80 per cent, and after 240 days, 83 per cent.

The proportion of the histidine nitrogen in the soil at the various times of sampling is about constant, with the exception of the 18-day sample.

#### *Arginine Nitrogen*

After 18 days 31 per cent of the arginine had vanished from the soil, while at the end of 148 days 83 per cent had gone. From the 148th to the 240th day, a period of 92 days, a gain in arginine nitrogen was observed. This may be due to nitrogen in the form of arginine, or nitrogen in the form of compounds which give the analytical reactions for arginine. It is nitrogen in organic compounds formed by the action of microorganisms, and is possibly in the form of proteins.

The relative amount of arginine nitrogen showed little fluctuation throughout the experiment and was a little greater at the end of the experiment.

*Amide Nitrogen*

The analysis of the figures for amide nitrogen brings out some interesting points. After 18 days there was an increase in amide nitrogen. It may be safely assumed that the compounds which this increase represents are acid amides, formed by the action of the microorganisms, existing in the soil either free or combined in the molecule of some new proteins contained in the protoplasm of organisms. That there was actually an increase in this form of nitrogen after 18 days was, however, unexpected, since it is well known that microorganisms, when grown in solutions of acid amides can use them for the building up of their protoplasm, and, furthermore, Jodidi (18) has shown that acid amides are very easily and quickly ammonified when placed in an agricultural soil. It was therefore expected before the results were obtained that the amide nitrogen would be one of the forms which would most quickly disappear from the soil. From Tables IV and VI it will be observed that this fraction disappears least completely and most slowly.

The question arose as to whether the soil used was capable of ammonifying acid amides. Consequently, 1 gm. of pure asparagine, one of the two acid amides considered to be present in the protein molecule, was added to 100 gm. of the air-dried Norfolk fine sandy loam to which no dried blood had been added. The soil was made to about a 10 per cent moisture content and allowed to stand for 4 days. On analysis for ammonia it was found that the soil had converted 73.4 mg. of asparagine nitrogen into ammonia nitrogen in this time, or in other words, the soil in 4 days had ammonified 39.3 per cent of the total asparagine nitrogen. This indicates that free acid amides in the soil would have been to a very large extent converted into ammonia during the 18 days of the experiment, and points unquestionably to the fact that the increase in this form of nitrogen is due to the synthetic action of the microorganisms in the building up of their own protoplasm.

After establishing the fact that the soil was capable of ammonifying acid amides it was decided to ascertain, if possible, if at any time previous to the first sampling period there occurred a decrease in amide nitrogen and at what time the increase in this form of nitrogen was first observable by the analytical methods. For this purpose some of the original mixture of soil and dried blood which had been shown to have undergone no change during storage, was taken and kept at a 10 per cent moisture content. Samples of this soil were taken at short intervals and analyzed for their content of free ammonia in the soil and ammonia in the hydrochloric acid extracts after hydrolysis. From these data it was possible to arrive at the amounts of amide nitrogen in the soil at the end of each sampling period. The results so obtained, together with the results already obtained upon amide nitrogen, are presented in Table VII.

TABLE VII  
AMIDE NITROGEN IN THE SOIL AT VARIOUS PERIODS

Time from the beginning of the experiment	Mg. of amide nitrogen per 100 gm. of oven-dried soil	Amide nitrogen expressed in percentages of hydrolyzable nitrogen in original soil
Original soil .....	57.14	7.008
2 days .....	59.77	7.329
3 days .....	60.15	7.363
5 days .....	8.75	1.606
6 days .....	34.31	4.207
7 days .....	38.42	4.628
8 days .....	60.42	7.408
13 days .....	57.48	7.020
18 days .....	61.39	7.515
20 days .....	60.72	7.110
44 days .....	49.13	6.025
86 days .....	44.38	5.429
148 days .....	28.27	3.454
240 days .....	26.38	3.222

The results show that during the second and third day there has been a slight increase in amide nitrogen. This must be considered as being due to the formation of protein material by the microorganisms in the form of their protoplasm, and since there has probably been little hydrolysis of the dried blood proteins at this time, the nitrogen necessary for this synthesis may have been derived from the free amino groups of the lysine of the native proteins of the dried blood, or from the free amino groups of lysine or other amino acids in albumoses which are also possibly present in the dried blood. On the fifth day the amide nitrogen had fallen from 60.15 mg. at the end of the third day to 8.75 mg., a loss of about 70 per cent of this form of nitrogen. Since the number of organisms in the soil is constantly increasing a portion of the amide nitrogen present in the soil at this time must be present in the form of proteins constituting the protoplasm of these organisms. It would appear, therefore, that practically all of the amide nitrogen of the dried blood proteins has been split off in 5 days, signifying a deep hydrolysis of these proteins.

The amide nitrogen increased from the fifth to the sixth day from 8.75 to 34.31 mg., the seventh day shows a further increase and on the eighth day the amount of amide nitrogen was about that present in the soil at the end of the third day; from the eighth to the twentieth day the amount of amide nitrogen, aside from small fluctuations, remained almost constant. This increase must be considered as being due to the synthetic action of the microorganisms in the formation of their protoplasmic proteins. In view of the fact that these proteins, in amount, must be much smaller than the proteins of the dried blood, but that the amide nitrogen content of the soil is even greater than the content of the original soil, it



would appear that the proteins formed by the microorganisms must be relatively rich in amide linkages.

The amide nitrogen of the soil during the time covered from the end of the first period of 18 days to the end of the experiment, 222 days, shows a loss of 57 per cent. This loss is by far smaller than any of the other forms of nitrogen. Since the amide nitrogen was practically all destroyed at the end of the fifth day and then amide nitrogen was synthesized, it would appear that the amide nitrogen built up by the action of microorganisms exists in proteins which are more resistant to the action of the microorganisms than were the proteins of the dried blood.

The figures in Table V show that the proportions of amide nitrogen in the soil increase up to the 86th day, when 22 per cent is reached as compared with 7 per cent in the original soil. The amount then drops off to 19 per cent.

In this connection it is extremely significant that the results obtained by Shorey (51), Lathrop and Brown (25), Jodidi (15, 16, 17), Kelly (20), and Potter and Snyder (41, 42) on hydrolyzing the nitrogenous compounds of soil and peats from this country and Hawaii, show amounts of amide nitrogen in the soils uniformly higher than are found by acid hydrolysis of animal or of vegetable proteins. These figures range between 16 and 30 per cent of the total hydrolyzable nitrogen of the soils. It has further been shown that some of these soils readily ammonify acid amides, indicating that the amide nitrogen exists in protein complexes and not as free acid amides. In the light of the present investigation these various analytical results seem to point to the presence in soils of considerable amounts of microorganismal proteins.

#### *Melanin and Non-amino Nitrogen*

Owing to the fact that the non-amino nitrogen varies so much throughout the experiment and that the melanins are so little understood, these two forms of nitrogen cannot be profitably discussed.

The results of this investigation are not in strict accord with those recently obtained by Kelly (21), who studied the decomposition of various sorts of organic matter in Hawaiian soils. He determined the amide, basic, and nonbasic nitrogen in casein, dried blood, soybean cake, cottonseed meal, linseed meal, cocoanut meal, globulin from cotton seed, and zein from maize, before and after the action of bacteria on these compounds in quartz sand to which a soil infusion has been added previous to incubation. His experiments covered from 3 to 8 days' decomposition and the amounts of organic matter used were about one-fourth the amount used in this investigation. He found that, with the exception of linseed meal and zein, the diamino nitrogen was converted into ammonia more rapidly than any other form of nitrogen. In the present investiga-

tion the conversion of the diamino nitrogen, arginine, histidine and lysine, into ammonia in 240 days amounts to 87 per cent, and the monoamino nitrogen 89 per cent. The causes for the differences in the results are probably not only the different experimental conditions but also a difference in the microörganismal flora, producing different types of the decomposition.

*Proteins in the soil at the end of the experiment*

From the results of the Van Slyke analysis evidence has been found to indicate that there is a formation of protein taking place in the soil in the course of the decomposition of protein materials, and that perhaps this new protein is somewhat resistant to decomposition. In order to determine whether or not soluble proteins are present in the soil after the decomposition had been proceeding 240 days the portion of the soil which remained was extracted with distilled water for several hours. The solution was then decanted from the soil and filtered. Tests for proteins or protein-like substances in the solution showed that such compounds had not been extracted by distilled water.

The soil was then treated with a 1 per cent solution of sodium hydroxide for 24 hours and this alkaline solution siphoned off from the soil. This solution was acidified with sulfuric acid and was filtered. To this acid filtrate 20 per cent phosphotungstic acid solution was added until precipitation had ceased, and after allowing the solution to stand for several hours until the precipitate had settled the precipitate was filtered off by suction and thoroughly washed with water acidulated with sulfuric acid. The phosphotungstic acid precipitate was suspended in cold distilled water and treated with an excess of barium hydroxide solution in order to free the protein material from the phosphotungstic and sulfuric acids. The excess of barium in the filtrate from the precipitate so formed was removed by carbon dioxide and the barium carbonate was filtered off. The solution was made just acid with dilute sulfuric acid and was boiled for a minute with a little barium carbonate and then filtered. A light straw-colored, turbid solution was obtained, which behaved in general like solutions of protein material. This solution was tested for the presence of proteins or protein-like substances. It gave precipitates with phosphotungstic, phosphomolybdic, tannic and picric acids, with mercuric chloride, silver nitrate, and copper acetate. The following tests for proteins were positive: Millon's reaction; Biuret test (reddish violet); Spiegler's ring test (weak); Robert's ring test (weak); Hopkins-Cole reaction, and Liebermann's reaction. Acetic acid and potassium ferrocyanide solution when added to the soil extract did not produce a precipitate, but a precipitate was formed when a solution of sodium chloride containing acetic acid was added. The protein material could be salted out by solid sodium chloride and by ammonium sulfate. A precipitate was formed on the addition of sufficient alcohol to make a 50 per cent

alcoholic solution, and the filtrate from this precipitate when treated with a large amount of absolute alcohol formed a further precipitate. A distinct cloudiness was formed in the solution on the addition of a half-saturated solution of ammonium sulfate. By these reactions the presence of proteins or protein-like substances in the soil is established. The exact class to which this protein material belongs could not be determined except by a more extended investigation. This established the fact that after a 240-day decomposition of dried blood in the soil, proteins, or protein-like complexes, not extractable by distilled water but soluble in dilute alkaline solution, were present in the soil. Whether they were proteins from the bodies of microorganisms in the soil, or whether they were residues from the dried blood which had until that time resisted decomposition by the microorganisms of the soil cannot be stated.

Of interest in this connection is the fact that Walters (60) has recently reported the isolation from a field soil of protein-like complexes which gave reactions for proteoses and peptones, bodies similar in nature and reaction to the compound here reported.

No attempt was made to isolate free amino acids from the soil after the decomposition period, since the quantities of soil were too small.

#### SUMMARY

The ammonification of the dried blood in the soil during the first 86 days was very rapid, after which time the amount of ammonia produced and the rate of ammonification decreased markedly until the end of the experiment. At the end of the experiment the rate of transformation of hydrolyzable nitrogen into ammonia nitrogen in the soil was but about 10 per cent of the rate observed after the decomposition had been proceeding for 18 days. During the 240 days of the experiment 79 per cent or more of the nitrogen of the dried blood proteins was converted into ammonia nitrogen.

The ammonia produced during the decomposition of the dried blood was derived from (1) the hydrolytic cleavage of the proteins of the dried blood, as evidenced by the rapid vanishing of the amide compounds from the soil during the first five days of the experiment; and (2) from the decomposition by the microorganisms of the products resulting from the hydrolytic cleavage of the proteins. Some of the ammonia produced during the first two or three days, when the hydrolysis of the proteins does not seem to have been very extended, may possibly have been due to the deamination of the  $\omega$ -amino group of the lysine in the native proteins of the dried blood. With the exception of the amide compounds lysine seems to have disappeared most rapidly and completely from the soil. The monoamino acids contributed about 89 per cent of their nitrogen to the formation of ammonia, and arginine and histidine each contributed about 83 per cent.

An analysis of the figures obtained by the Van Slyke method points to the generation of new protein materials in the soil. This is indicated by (1) the unequal loss of monoamino acids and hydrolyzable nitrogen from the soil during the early stages, (2) by an increase in amide nitrogen during the early stages, (3) by an increase in histidine nitrogen during the early stages, (4) by an increase in arginine nitrogen during the later stages, and (5) by an increase in lysine nitrogen during the later stages.

This new form of protein seems to be more resistant to the action of the microorganisms than were the proteins of the dried blood, since the amide compounds of the dried blood vanished very largely from the soil in 5 days but the amide compounds produced in the soil decreased only to the extent of 57 per cent during the remaining 222 days of the experiment, and also since the lysine of the dried blood almost entirely disappeared from the soils during the first 86 days of the experiment, but during the last 154 days of the experiment a continual increase in this form of nitrogen was observed.

Protein-like substances, non-extractable by distilled water but extractable by 1 per cent sodium hydroxide solution, were isolated from the soil after the dried blood had decomposed for 240 days. Whether these were residues from the dried blood which had until this time resisted decomposition by the microorganisms or were proteins produced by the microorganisms cannot be stated.

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# THE OXIDATION OF SULFUR IN SOILS AS A MEANS OF INCREASING THE AVAILABILITY OF MINERAL PHOSPHATES<sup>1</sup>

By

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The increase in the cost of phosphate which occurred in the fall of 1915 led the senior author of this paper to suggest the use of elementary sulfur for the purpose of rendering soluble inert phosphates. When this suggestion was made toward the end of 1915, European and American investigators had already shown that elementary sulfur is readily oxidized in the soil, and that such oxidation is largely, if not entirely, the result of biological activities. A bibliography on the subject of sulfur oxidation in soils is given elsewhere;<sup>2</sup> in this place it need be stated, merely, that environmental conditions play an important rôle in the activities of sulfur oxidizing microorganisms. Apart from the abundant supply of oxygen which is obviously essential in any oxidizing reaction, moisture and the amount and quality of the organic matter are factors of direct significance. Moreover, the numbers and physiological efficiency of the organisms themselves are always of prime importance. As will be shown in the following pages, the oxidation reaction becomes gradually more intense, an indication that the sulfofying flora becomes more effective in response to a favorable environment.

It appears that there is a strong analogy between nitrification and sulfofication. In both cases the reaction is accomplished by obligate aerobes. In both instances a large amount of readily decomposable organic matter is undesirable, for the organisms prefer a medium whose organic matter had become at least partly mineralized. In both instances a relatively large amount of energy is made available in the oxidation process. In both instances an efficient oxidizing flora is developed gradually. Indeed, it is even possible that nitrification and sulfofication may be brought about by the same species of bacteria. To be sure, pure cultures of sulfofying bacteria which oxidize sulfur rather than hydrogen

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<sup>2</sup> "Sulfur Oxidation in Soils as Affecting the Availability of Mineral Phosphates," a thesis submitted by Harry C. McLean to the Faculty of Rutgers College for the Degree of M.Sc., May, 1916.



sulfide as the initial compound have not yet been isolated, and an intimate knowledge of them is still to be gained. Nevertheless, our present knowledge is sufficiently definite to warrant the statement that compost heaps, as well as cultivated fields, may be so treated as to provide a congenial environment for sulfofying bacteria. Under such favorable environments these organisms may be utilized for producing quantities of sulfuric acid sufficient for the transformation of large amounts of tricalcic into dicalcic or even monocalcic phosphate. There is every reason to think that the method here suggested is capable of the widest application in agricultural practice. When properly employed it should enable the farmer and the gardener to secure available phosphorus at a low cost and to provide at the same time enormous numbers of active oxidizing bacteria. The method proposed here is also an argument for the return to the practice of composting so prominent in the agriculture of fifty years ago.

Soil microbiology has made sufficient progress to justify the claim that the lowered productive capacity of much of our land is due to the neglect of the microbiological machinery of the soil. By returning to the practice of composting we shall again make possible the frequent addition to our land of very large numbers of desirable bacteria. But what is even more important, we shall learn to appreciate vividly that crops depend on microorganisms for the elaboration of available plant-food, and that a defective soil environment must depress crop yields. We shall strive, then, to make our fields approach the condition of a compost heap by the intelligent use of lime, green manures and chemical manures.

The present paper is in the nature of a preliminary communication. Other communications on sulfofication and sulfofying bacteria will be forthcoming. Meanwhile, proof is submitted in the following pages that large quantities of citrate-soluble phosphates may be produced in soils or soil mixtures to which there has been added finely divided sulfur and finely ground phosphate rock or other finely divided tricalcic phosphate. The commercial possibilities of the proposed method hardly need elaboration.

The sulfofication experiments recorded here were carried out in three media, one of them pure sea sand, one a tenacious red silt loam, and the third a medium loam commonly designated as Sassafras loam. The red silt loam contained 0.297 per cent  $P_2O_5$ ; 10.14 per cent  $Fe_2O_3$  and  $Al_2O_3$ , and 3.04 per cent organic carbon. The Sassafras loam contained 0.1950 per cent  $P_2O_5$ ; 5.39 per cent  $Fe_2O_3$  and  $Al_2O_3$ , and 1.07 per cent of organic carbon. It will be noted that the red silt loam was quite rich in organic carbon and phosphorus. This was due largely to composted manure which had been mixed with the soil preparatory to its use in the greenhouse for the growing of roses and carnations.

In arranging for the sulfonation experiments, quantities of the three soil media described above were thoroughly air-dried and passed through a sieve containing ten meshes to the lineal inch. The ground phosphate rock or "floats" employed in this experiment was derived from brown Tennessee rock containing 30 per cent of  $P_2O_5$  and fine enough to pass to the extent of 95 per cent through a sieve which had 10,000 meshes to the square inch.

As shown in the accompanying table, 100-gm. quantities of soil in glass tumblers were used as the medium for sulfonation. The mixtures of rock phosphate and sulfur were made in accordance with the plan given and water was added up to 50 per cent of the water-holding capacity of the soil. In order to insure proper inoculation and to furnish at least some mineral food to the bacteria in the sand cultures, there was added to the mixture in each tumbler 10 c.c. of a soil infusion prepared by shaking for 10 minutes 100 gm. of fertile soil with 200 c.c. of a synthetic culture medium which contained no phosphorus. The tumblers and contents were then weighed and the weights recorded. From time to time the tumblers were reweighed and the moisture that had been lost by evaporation was restored. During the progress of the experiment the tumblers, covered with Petri dish covers, were kept in a dark closet at a temperature of 22° C. to 24° C.

Determinations of acidity, and of citrate-soluble and water-soluble phosphoric acid were made at the end of each week during the first eight weeks of the experiment. After that the determinations were made at intervals of two weeks. The results secured are recorded in Table I.

TABLE I

THE INFLUENCE OF SULFONATION ON THE ACCUMULATION OF AVAILABLE PHOSPHORIC ACID IN 15 WEEKS

Tumbler No.	Soil Medium and Treatment	$P_2O_5$ soluble in Ammonium Citrate		$P_2O_5$ soluble in Water	
		Average mg.	Inc. over ck. mg.	Average mg.	Inc. over ck. mg.
1, 2	Sand, 5 gm. Sulfur.....	33.80	.....	.....	.....
3, 4	Sand, 15 gm. Floats.....	116.47	.....	15.10	.....
5, 6	Sand, 5 gm. Sulfur, 15 gm. Floats....	400.68	284.21	185.23	170.13
7, 8	Red Silt Loam, 5 gm. Sulfur.....	160.10	.....	.....	.....
9, 10	Red Silt Loam, 15 gm. Floats.....	138.77	.....	30.10	.....
11, 12	Red Silt Loam, 5 gm. Sulfur, 15 gm. Floats .....	1982.40	1843.63	999.56	969.46
13, 14	Sassafras Loam, 5 gm. Sulfur.....	101.40	.....	.....	.....
15, 16	Sassafras Loam, 15 gm. Floats.....	168.50	.....	18.40	.....
17, 18	Sassafras Loam, 5 gm. Sulfur, 15 gm. Floats .....	867.30	698.80	178.11	159.71

It is clearly shown by the amount of citrate-soluble as well as water-soluble phosphoric acid found at the end of 15 weeks that there was a very pronounced oxidation of the sulfur added and that the resulting sulfuric acid had reacted with the tricalcic phosphate. It is also apparent that the character of the soil employed, particularly as regards its mechanical and chemical composition, played an important part in stimulating or retarding the activities of the sulfofying bacteria. For instance, the sand contained at the end of 15 weeks 400.68 mg. of citrate-soluble phosphoric acid, and 185.23 mg. of water-soluble phosphoric acid where 5 gm. of sulfur and 15 gm. of floats were employed. Under the same conditions there were found in the red silt loam 1982.40 mg. of citrate-soluble and 999.56 mg. of water-soluble phosphoric acid. In the Sassafras loam the oxidation processes were not as intense as in the red silt loam, but much more intense than in the sand. However, in order to appreciate fully the influence of the soil medium on the rate of sulfur oxidation, one must compare the data secured at the end of each week within the first eight weeks, and at the end of each subsequent two weeks for seven weeks more. The rate of accumulation of sulfuric acid as affected by the aeration of the medium and the reaction of the acid formed with the basic material present will then be more clearly understood. A clearer understanding will also be had of the fact that the intensity of sulfur oxidation gradually gathers momentum under conditions favorable for the development of a strong sulfofying flora. Such favorable conditions of necessity encourage a more rapid multiplication of the microorganisms and probably also the establishment of the most effective strains or species of sulfofiers. It is possible also that associative action between sulfofying and non-sulfofying microorganisms plays a part in determining the type and degree of sulfur oxidation. The data presented in Table II serve to show the influence of each soil on the accumulation of acid.

In the sand the accumulation of acid in 15 weeks was equivalent to 100 c.c. of N/50 potassium hydrate. This occurred in the soil portions to which sulfur had been added. On the other hand, the amounts of acid found in the soil portions to which no sulfur had been added were quite small. It is interesting to note, at the same time, that, in the soil portions which had received additions of both floats and sulfur, the accumulation of acid reached, at the end of the tenth week, an equivalent of 338 c.c. of N/50 potassium hydrate. It seems, therefore, that, the presence of the tricalcic phosphate did not decrease the accumulation of acid up to a certain point. Possibly the presence of available phosphate stimulated the activities of the sulfur oxidizing bacteria so that actually more sulfuric acid was produced than in the soil portions to which no phosphate had been added. It should be noted, also, that the maximum accumula-

TABLE II  
THE ACCUMULATION OF ACID AND AVAILABLE PHOSPHATES IN THREE SOIL  
MEDIA IN A PERIOD OF 15 WEEKS

## SAND

Time	Additions							
	None		5 gm. Sulfur		15 gm. Floats		5 gm. Sulfur 15 gm. Floats	
	Acidity cc. N/50 KOH	P <sub>2</sub> O <sub>5</sub> mg.	Acidity cc. N/50 KOH	P <sub>2</sub> O <sub>5</sub> mg.	Acidity cc. N/50 KOH	P <sub>2</sub> O <sub>5</sub> mg.	Acidity cc. N/50 KOH	P <sub>2</sub> O <sub>5</sub> mg.
Beginning .....	6.00	34.58	7.25	35.06	5.75	136.63	7.50	139.04
End of 1st week ....	6.00	33.85	12.00	35.06	6.00	169.26	9.50	171.68
End of 2nd week ....	.....	.....	.....	.....	7.25	139.00	21.50	180.38
End of 3rd week ....	8.00	32.64	34.50	33.37	7.50	177.72	46.50	160.80
End of 4th week ....	.....	.....	.....	.....	8.25	187.39	143.00	234.55
End of 5th week ....	6.50	27.81	41.50	28.23	6.50	168.05	174.50	224.62
End of 6th week ....	.....	.....	.....	.....	7.75	122.10	177.00	272.02
End of 7th week ....	7.50	25.39	58.00	24.18	9.50	136.62	215.00	258.73
End of 8th week ....	.....	.....	.....	.....	7.00	136.70	208.00	262.87
End of 10th week ...	7.50	31.43	96.00	34.52	7.50	136.62	338.00	337.31
End of 12th week ....	.....	.....	96.00	.....	7.00	138.20	336.00	390.62
End of 15th week ...	.....	.....	100.00	33.80	.....	116.47	328.50	400.68
Increase .....	.....	.....	92.75	.....	.....	.....	321.00	261.64

## RED SILT LOAM

Beginning .....	2.50	111.95	5.00	105.18	4.25	143.39	5.50	142.66
End of 1st week ....	2.00	111.71	8.75	96.72	4.25	166.84	11.50	166.44
End of 2nd week ....	.....	.....	.....	.....	3.00	176.51	18.75	203.11
End of 3rd week ....	4.75	123.11	42.75	120.90	6.25	191.02	89.00	272.75
End of 4th week ....	.....	.....	.....	.....	6.00	206.74	224.00	227.29
End of 5th week ....	2.75	79.79	137.00	100.34	2.25	166.13	209.00	230.92
End of 6th week ....	.....	.....	.....	.....	6.00	148.21	281.00	258.73
End of 7th week ....	5.25	83.42	310.60	103.49	6.00	157.17	324.00	253.89
End of 8th week ....	.....	.....	.....	.....	5.50	160.79	352.00	320.38
End of 10th week ....	6.50	105.18	4404.00	154.75	5.50	153.54	616.00	340.94
End of 12th week ...	.....	.....	4560.00	.....	.....	.....	596.00	396.88
End of 15th week ...	.....	.....	4700.00	160.10	.....	138.77	710.00	1982.40
Increase .....	.....	.....	4695.00	.....	.....	.....	704.50	1839.74

## SASSAFRAS LOAM

Beginning .....	6.00	79.79	6.50	83.42	5.55	155.96	7.50	154.27
End of 1st week ....	9.50	77.37	11.25	94.30	10.00	162.01	10.00	164.91
End of 2nd week ....	.....	.....	.....	.....	10.00	191.01	11.50	222.46
End of 3rd week ....	6.00	120.90	33.50	125.74	6.25	171.68	107.50	209.16
End of 4th week ....	.....	.....	.....	.....	7.00	180.14	360.50	269.61
End of 5th week ....	7.50	99.14	454.50	103.97	5.25	165.63	353.00	342.15
End of 6th week ....	.....	.....	.....	.....	6.50	155.75	421.00	406.22
End of 7th week ....	7.00	71.33	597.00	89.47	10.50	158.38	485.00	339.73
End of 8th week ....	.....	.....	.....	.....	8.50	175.67	478.00	508.96
End of 10th week ...	7.00	96.72	1240.00	100.35	8.50	158.86	660.00	518.66
End of 12th week ...	.....	.....	1300.00	.....	.....	.....	637.00	655.20
End of 15th week ...	.....	.....	1440.00	101.40	.....	168.50	570.25	867.30
Increase .....	.....	.....	1433.50	.....	.....	.....	562.75	713.03

tion of acid was found at the end of the tenth week. After that, the amount of acid found in the soil portions similarly treated was practically constant.

In the case of the red silt loam there was an increase of acid in the soil portions to which sulfur alone was added up to the end of the fifteenth week. At that time the total amount of acid found was equivalent to 4700 c.c. of N/50 potassium hydrate. The most striking increase was made between the seventh and the tenth week, when the acid increased from an equivalent of 310.60 c.c. of N/50 potassium hydrate to an equivalent of 4404 c.c. of N/50 potassium hydrate. Beyond that, the increase was but slight. When both sulfur and floats were added to the soil portions, there was a greater amount of acid accumulated in the first seven weeks of the experiment than there was in the corresponding soil portions to which sulfur alone was added. On the other hand, we find that, at the end of the tenth week, the total amount of acid found in the soil portions to which both sulfur and floats were added was equivalent to 646 c.c. of N/50 potassium hydrate as against 4404 c.c. in the soil portions to which sulfur alone was added. Beyond that point there was comparatively little change in the acidity of the soil portions that had received additions of both sulfur and floats.

In the case of Sassafras loam soil, there was also a very marked accumulation of acid in the soil portions which had additions of sulfur only. The increase was gradual up to the end of the fifteenth week. At that time it was equivalent to 1440 c.c. of N/50 potassium hydrate. Where both floats and sulfur were used, the increase in acidity was more marked at first than it was in the corresponding portions to which sulfur alone was added. Later on, however, the accumulation in the sulfur portions became greater than in the sulfur-floats soil portions. Thus, at the end of the tenth week, the sulfur-floats portions contained an equivalent of 660 c.c. N/50 potassium hydrate as against 1240 c.c. of N/50 potassium hydrate. After the end of the tenth week there was, if anything, a slight decline in the amount of acid found in the sulfur-floats portions.

The amounts of available phosphoric acid found in the three types of soil media indicate that the sulfuric acid produced in the oxidation of the sulfur had reacted with the tricalcic phosphate. Reference has already been made to the amounts of available phosphoric acid found at the end of 15 weeks in each of the three soil media employed. It need only be added here that, in the case of the sand, the total amount of available phosphoric acid at the end of the tenth week was equivalent to 337.31 mg. Beyond that the increase was relatively small. In the case of the red silt loam, the amount of available phosphoric acid at the end of the tenth week was equivalent to 340.94 mg. The striking increase came from the twelfth to the fifteenth week, when the total amount of

phosphoric acid found was equivalent to 1982.40 mg. In the Sassafras loam the increase in the amount of available phosphoric acid was more gradual. At the end of the tenth week an equivalent of 518.66 mg. of phosphoric acid was found in each soil portion which had received additions of both floats and sulfur, while, at the end of the fifteenth week, the corresponding amount found was 867.30 mg. It would seem, therefore, that the oxidation of sulfur in soils of different types is intimately dependent upon the number and physiological efficiency of sulfofying bacteria. These in their turn are readily affected by the mechanical and chemical composition of the soil medium employed.

#### SUMMARY

1. Elementary sulfur is readily oxidized in soils containing sulfofying bacteria and offering favorable conditions for the development of these organisms.
2. The oxidation of sulfur in soils may lead to the accumulation of large quantities of sulfuric acid.
3. The sulfuric acid formed in the oxidation of sulfur by bacteria readily reacts with basic substances.
4. Tricalcic phosphate, when added to soils or soil mixtures in which sulfofication is active, may react with the sulfuric acid formed, and may then furnish available phosphoric acid to crops.
5. The facts recorded above justify the claim that compost heaps in which sulfofication is active may be utilized for the production of available phosphoric acid out of insoluble phosphates.



# THE EFFECT OF SOIL REACTION ON AMMONIFICATION BY CERTAIN SOIL FUNGI<sup>1</sup>

By

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## INTRODUCTION

The development of the science of soil biology has been marked, from time to time, by the direction of attention towards different groups of microorganisms, the bacteria, protozoa and fungi. Despite the more or less tacit understanding that fungi have various functions to perform in the soil, they have received comparatively little consideration at the hands of the soil biologist. Without assuming any exaggerated importance in their behalf, there is good reason to believe that they are a significant factor in the decomposition which takes place in soils. It appears that fungi are particularly active in the early stages of the decomposition of both nitrogenous and non-nitrogenous organic matter. It has previously been pointed out (11) that many soil fungi have a high ammonifying efficiency. This fact may be interpreted as indicating that this group of microorganisms has a bearing on the problems of soil fertility.

Obviously enough, the environmental conditions are of paramount importance in influencing their physiological activities, and principal among these is reaction. A general consideration of the occurrence, distribution and activities of soil fungi is not sufficiently pertinent to the subject at hand to necessitate any further review than has already appeared (6, 23, 24).

However, it is of interest to note that Fischer (9), Oudenmans and Koning (16), Ramann (18), and Faelli (7), have reported the occurrence of fungi in acid soils having a high organic content. Hall, Miller and Gimingham (10) found that the decline in fertility of plots which had become acid through continued use of ammonium sulfate could be attributed to the repression of the normal bacterial activities of the soil and the encouragement of molds. Marchal (15) also states that soils having a weakly alkaline or neutral reaction have relatively few fungi. Fellers (8) found that heavy soils gave the highest fungi counts on certain agar media having a reaction of 1 to 1½ per cent acidity (HCl). Alkaline media were injurious to the growth of these organisms. *Asper-*

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*gillus niger*, *Cladosporium epiphyllum*, *Penicillium viridicatum* and *Trichodermae* all attained their maximum growth on acid media, while alkalinity proved distinctly unfavorable. This investigator notes that all the fungi studied appeared to have a wide range of reaction tolerance.

In general it is an accepted fact that neutral to acid soils are most congenial to the development of soil fungi, while an alkaline condition is for the most part, unfavorable. There is then something of a balance maintained between the number of fungi and bacteria as determined by the reaction of the soil. Or, in other words, where acidity prevails, there is a tendency for the bacteria to diminish and for the fungi to increase accordingly, while with soils having an alkaline reaction, there would be a relatively greater number of bacteria than fungi. Since it has been shown by the investigators previously mentioned that soil fungi have marked ability in producing ammonia from organic nitrogenous materials, it may reasonably be inferred that under conditions which are unfavorable to the development of great numbers of bacteria, the soil fungi would assume a considerable degree of importance in maintaining the fertility of soils. Thus in acid soils the production of ammonia by soil fungi would compensate for the reduced bacterial activities.

The purpose of the following experimentation was to determine the effect of varying soil reaction upon the ammonification of organic nitrogenous materials by certain soil fungi.

#### METHODS

Two-hundred-c.c Erlenmeyer flasks containing 100-gm. portions of two soils, designated as Norfolk sandy loam, and Penn clay loam, respectively, were employed throughout this work. Dried blood and cottonseed meal in quantities equivalent to 155 mg. N. were used as sources of organic nitrogenous material to be ammonified. The soil after being treated was thoroughly mixed by shaking in a receptacle adapted to that purpose (12). With Norfolk sandy loam the series of flasks containing dried blood received 16.7 c.c. of water, while the series containing cottonseed meal received 20.5 c.c. With Penn clay loam the series containing dried blood received 29.7 c.c. of water, while the series containing cottonseed meal received 33.5 c.c. Thus in all cases the soil was kept at a moisture content very slightly above the optimum. Proper deduction was made in all cases for inoculum or any other liquid added. After the reaction of the soil had been altered according to the plan to be discussed presently, the flasks containing the soil were placed in the autoclave for 15 minutes at 15 pounds pressure. (This process was responsible for a loss of approximately 2 c.c. of moisture per flask.) Upon cooling, 1 c.c. of spore-suspension of the desired organism, prepared and counted according to the method described in detail in Part I (11), was inoculated into the soil, and the flasks incubated at 20° to 22° C. for 7 days, except

in the case of the *Penicillium*, where a 12-day period was found to be necessary. At the end of this time the contents of the flasks were examined for bacterial contamination by plating a small portion of the soil on Lipman and Brown's synthetic agar (13). (This practice was later discontinued, since the variation between duplicate determinations furnished an adequate criterion, in the few cases where contamination occurred.) The soil was then transferred to copper flasks, the ammonia distilled according to the magnesium oxide method and titrated with N/10 acid and alkali.

The fungi used were isolated in pure pedigree culture from soil on the College Farm and were tentatively identified as *Rhizopus nigricans*, Ehrenberg; *Zygorrhynchus Vuilleminii*, Namyslowski; and *Penicillium* sp. 10. These three organisms have been found to be present in soils by most investigators who have isolated soil fungi (24). (This particular *Penicillium* is to be considered as representative of a group of green soil *Penicillia*.) The organisms under consideration are sufficiently different in their morphology and physiological activities to offer some basis for generalization. The following work, however, must be considered under the limitations necessitated by studies with pure cultures, namely: it is questionable whether these organisms would act in the same manner when associated with other fungi, or even bacteria. Secondly, the soil as a culture medium, in the process of sterilization, undergoes certain changes which might be responsible for peculiarities not permitting of an absolute correlation with actual field conditions (5). The investigation under discussion may best be divided into two sections. The first part deals with the effect of soil reaction on ammonification by fungi, where the reaction of the soil has been altered by the addition of normal solutions of hydrochloric acid or sodium hydroxide. In the second part, the reaction of the soil has been altered by the addition of calcium carbonate (c. p.) or a normal solution of sulfuric acid.

## I

### *The Effect of Soil Reaction on Ammonification by Certain Soil Fungi, When the Reaction Has Been Altered by Additions of Normal Solutions of HCl or NaOH*

Since the problem at hand is chemical in its nature, it seemed advisable to alter the reaction of the soil by materials which would not function as food for the fungi concerned. Therefore HCl and NaOH were desirable for this purpose, as it is an established fact that none of the ions present in the above chemicals is an essential nutrient for fungi. Furthermore, in order to ensure against the possibility of either the Na or the Cl ions causing undue stimulation or depression, a solution of NaCl (3 N) was added to all the flasks in an amount approximately equivalent to the highest Na or Cl application.

Clark (4) states that the Cl ion is relatively harmless to molds and that OH is more toxic than ionic H to *Penicillium glaucum*. So far as the writer has been able to determine there is no record other than the work of McLean and Wilson (14) of any systematic experiments dealing with the alteration of reaction in the soil as affecting either the growth or the physiological activities of soil fungi.

Thom (22) reports studies with *Penicillia* where normal NaOH and normal lactic acid were added to tubes containing 10 c.c. of medium neutral to phenolphthalein. It was found that the range of tolerance in the species studied was from 2 c.c. of NaOH per 10 c.c. of medium, to 5 c.c. of acid per 10 c.c. of medium. Within this extreme range most species are more closely restricted. Very few species grow to any degree in plates alkaline to phenolphthalein. Of common green species but few fruited freely in alkali as strong as N/10. Nearly all grew best between the neutral point and an acidity approximately equal to N/10. He further suggests that this inhibiting effect of acidity varies with the species and the kind of acid used. Stevens (21) finds that the *Penicillium* spores which he studied grew in N/50 HCl and N/50 H<sub>2</sub>SO<sub>4</sub>, also in 2 N NaCl, solutions, but failed to grow in N/40 NaOH. Traaen (23) found that with most of the fungi he studied, N/150 to N/50 acid inhibited growth. *Trichoderma* appeared to be more resistant. He states further that HCl was less toxic than HNO<sub>3</sub>. Beck (1) found that fungi which grew sparingly in N/10 HCl did not affect the titre of the acid.

It could hardly be expected that small amounts of acid or alkali would prove as toxic in soil containing a considerable supply of moisture, as in solutions such as noted by these investigators. In this experiment the Norfolk sandy loam used (for *Rhizopus* and *Zygorrhynchus*) had a lime requirement of 400 pounds of CaO per acre, on the basis of 3,000,000 pounds of soil per surface 6 2/3 inches, while that of the Penn clay loam was 1,700 pounds of CaO per acre on the basis of 2,700,000 pounds of soil per surface 6 2/3 inches. In order to approximate, as closely as possible, actual field conditions, the treatment in both the dried blood and cottonseed meal series consisted of increasing the acidity of the soil from the neutral point, in amounts equivalent to 1,000 pounds of CaO per acre, up to 4,000 pounds. Similarly, the soil was made basic by the addition of CaO up to 4,000 pounds per acre. (In the remainder of this discussion, such conditions will be referred to as "alkaline.") Most normal soils fall quite readily within these limits.

Sufficient normal HCl or NaOH was added to bring about the desired reaction. Thus in the column marked "Treatment" in Table I, "Acid  $\approx$  400 lbs. CaO" represents the original reaction of the sandy soil, while to obtain an acidity of 1,000 pounds of CaO per acre, it is evident that an addition of acid equivalent to 600 pounds CaO per acre was required, or 0.72 c.c. HCl (N/1). For an acidity of 2,000 pounds per acre, 1.92 c.c.

HCl was required, i. e., 1.2 c.c. of normal acid or alkali is equivalent to 1,000 pounds CaO per acre. Thus to obtain an alkalinity of 1,000 pounds CaO per acre, it was necessary to add 1.68 c.c. NaOH (N/1). Five-tenths of a cubic centimeter of NaCl (3 N) solution was added to all flasks.

The release of ammonia from the soil as a result of the highest application of acid or alkali did not exceed 1 to 2 mg. N, and therefore this amount was not deducted from the checks. In the following discussion, continued reference will be made to "1,000 pounds acid," "1,000 pounds alkaline," etc. It is to be assumed that "pounds of CaO per acre" is understood. The results recorded in the subsequent tables represent the two more closely agreeing determinations of an experiment carried out in triplicate.

TABLE I  
THE EFFECT OF REACTION ON RHIZOPUS NIGRICANS IN NORFOLK SANDY LOAM  
(HCl—NaOH)

No.	Organic Matter	Treatment	NH <sub>3</sub> accumulated in			Increase over check Mg. N.
			Mg. N.	Mg. N.	Av. Mg. N.	
1-2	155 mg. N. Dried Bl'd	Check . . . . .	3.39	3.55	3.47	.....
3-4	"	Soil L. R. $\approx$ 400 lbs. CaO	30.70	30.70	30.70	27.23
5-6	"	Acid $\approx$ 1000 lbs. CaO....	42.46	39.40	40.93	37.46
7-8	"	Acid $\approx$ 2000 lbs. CaO....	42.54	43.88	43.21	39.74
9-10	"	Acid $\approx$ 3000 lbs. CaO....	19.45	21.18	20.31	16.84
11-12	"	Acid $\approx$ 4000 lbs. CaO....	13.49	8.44	10.96	7.49
13-14	"	Neutral . . . . .	31.95	31.24	31.58	28.11
15-16	"	Alk. $\approx$ 1000 lbs. CaO....	26.08	24.36	25.22	21.75
17-18	"	Alk. $\approx$ 2000 lbs. CaO....	19.88	19.88	19.88	16.41
19-20	"	Alk. $\approx$ 3000 lbs. CaO....	10.70	13.91	12.31	8.84
21-22	"	Alk. $\approx$ 4000 lbs. CaO....	13.49	14.73	14.11	10.64
23-24	155 mg. N. Cottonseed	Check . . . . .	3.69	3.69	3.69	.....
25-26	Meal	Soil L. R. $\approx$ 400 lbs. CaO	42.97	43.38	43.17	39.48
27-28	"	Acid $\approx$ 1000 lbs. CaO....	39.52	41.05	40.28	36.59
29-30	"	Acid $\approx$ 2000 lbs. CaO....	33.18	25.70	29.44	25.75
31-32	"	Acid $\approx$ 3000 lbs. CaO....	27.06	26.15	26.60	22.91
33-34	"	Acid $\approx$ 4000 lbs. CaO....	26.10	23.83	24.96	21.27
35-36	"	Neutral . . . . .	36.83	39.21	38.02	34.33
37-38	"	Alk. $\approx$ 1000 lbs. CaO....	29.96	30.95	30.45	26.76
39-40	"	Alk. $\approx$ 2000 lbs. CaO....	25.53	27.83	26.65	22.96
41-42	"	Alk. $\approx$ 3000 lbs. CaO....	24.08	24.85	24.46	20.77
43-44	"	Alk. $\approx$ 4000 lbs. CaO....	6.82	7.82	7.32	3.63

An examination of Table I, which gives the effect of soil reaction on ammonification by *Rhizopus nigricans* in Norfolk sandy loam reveals the fact that in the dried blood series there is a sharp increase in ammonia accumulated where the reaction of the soil is 1,000 pounds acid compared with an acidity of but 400 pounds. There is, further, a slight increase in ammonia as the acidity is increased to 2,000 pounds, and thereafter a striking decrease is noted as the acidity is raised to 3,000 and 4,000 pounds, respectively.

In Plate I it will be seen that the mycelial growth is directly correlated with the curve of ammonia accumulation. Where the soil is made alkaline there is a gradual decline in ammonia from the neutral point with each successive application equivalent to 1,000 pounds CaO per acre until 3,000 pounds is reached. Four thousand pounds gives practically the same yield as 3,000.

Plate II, illustrating the effect of alkalinity on mycelial growth, correlates with and may be considered as the graphic representation of the curve of ammonia accumulation in this series. In the series where cottonseed meal was used as a source of organic matter, it will be seen that so far as ammonia accumulation is concerned there is a gradual decrease with successive 1,000-pound applications of acidity. Plate III, however,

TABLE II  
THE EFFECT OF REACTION ON RHIZOPUS NIGRICANS IN PENN CLAY LOAM  
(HCl—NaOH)

No.	Organic Matter	Treatment	NH <sub>3</sub> accumulated in			Increase over check Mg. N.
			Mg. N.	Mg. N.	Av. Mg. N.	
	155 mg. N. Dried Bl'd	Check .....	4.23	4.96	4.60	.....
89-90	"	Soil L. R. $\approx$ 1700 lbs. CaO	21.27	20.87	21.07	16.47
91-92	"	Acid $\approx$ 400 lbs. CaO....	23.81	23.81	23.81	19.21
93-94	"	Acid $\approx$ 1000 lbs. CaO....	23.23	22.79	23.01	18.41
95-96	"	Acid $\approx$ 2000 lbs. CaO....	20.76	22.34	21.55	16.95
97-98	"	Acid $\approx$ 3000 lbs. CaO....	17.59	15.44	16.57	11.97
99-100	"	Acid $\approx$ 4000 lbs. CaO....	21.61	19.70	20.66	16.06
101-102	"	Neutral .....	22.05	21.61	21.83	17.23
103-104	"	Alk. $\approx$ 1000 lbs. CaO....	21.76	22.20	21.98	17.38
105-106	"	Alk. $\approx$ 2000 lbs. CaO....	17.93	17.49	17.71	13.11
107-108	"	Alk. $\approx$ 3000 lbs. CaO....	18.72	18.72	18.72	14.12
109-110	"	Alk. $\approx$ 4000 lbs. CaO....	16.76	16.76	16.76	12.16
111-112	"					
	155 mg. N. Cottonseed Meal	Check .....	5.21	4.99	5.10	.....
113-114	"	Soil L. R. $\approx$ 1700 lbs. CaO	22.93	23.37	23.15	18.05
115-116	"	Acid $\approx$ 400 lbs. CaO....	25.58	25.43	25.51	20.41
117-118	"	Acid $\approx$ 1000 lbs. CaO....	27.05	27.34	27.20	22.10
119-120	"	Acid $\approx$ 2000 lbs. CaO....	25.87	26.17	26.02	20.92
121-122	"	Acid $\approx$ 3000 lbs. CaO....	21.02	19.99	20.51	15.41
123-124	"	Acid $\approx$ 4000 lbs. CaO....	17.93	19.55	18.79	13.69
125-126	"	Neutral .....	28.37	26.46	27.42	22.32
127-128	"	Alk. $\approx$ 1000 lbs. CaO....	26.17	25.28	25.73	20.63
129-130	"	Alk. $\approx$ 2000 lbs. CaO....	24.55	23.52	24.04	18.94
131-132	"	Alk. $\approx$ 3000 lbs. CaO....	21.76	20.87	21.32	16.22
133-134	"	Alk. $\approx$ 4000 lbs. CaO....	27.93	26.02	26.98	21.88
135-136	"					

exhibits a gradual increase in mycelial growth from 400 to 2,000 pounds acidity, followed by a sharp decrease as a result of an application beyond this point. Thus in the present instance there is no correlation between mycelial growth and ammonia accumulation, the results regarding the latter suggesting the following interpretation. It is to be expected that the greater the mycelial growth the greater is the amount of nutrients consumed in its formation. Since ammonia accumulation must be con-

sidered as a process involving the concomitant factors of production and consumption of ammonia, it may be readily conceived how it was possible for more ammonia to have been produced with an acidity of 2,000 compared with 400 pounds, but that the ammonia thus produced was consumed by the fungus in mycelial development, in such a manner as to yield a smaller quantity of ammonia. It must be borne in mind that in biological studies of this nature, it is impossible to anticipate entirely coherent or concordant results at all times. The fact that a living organ-

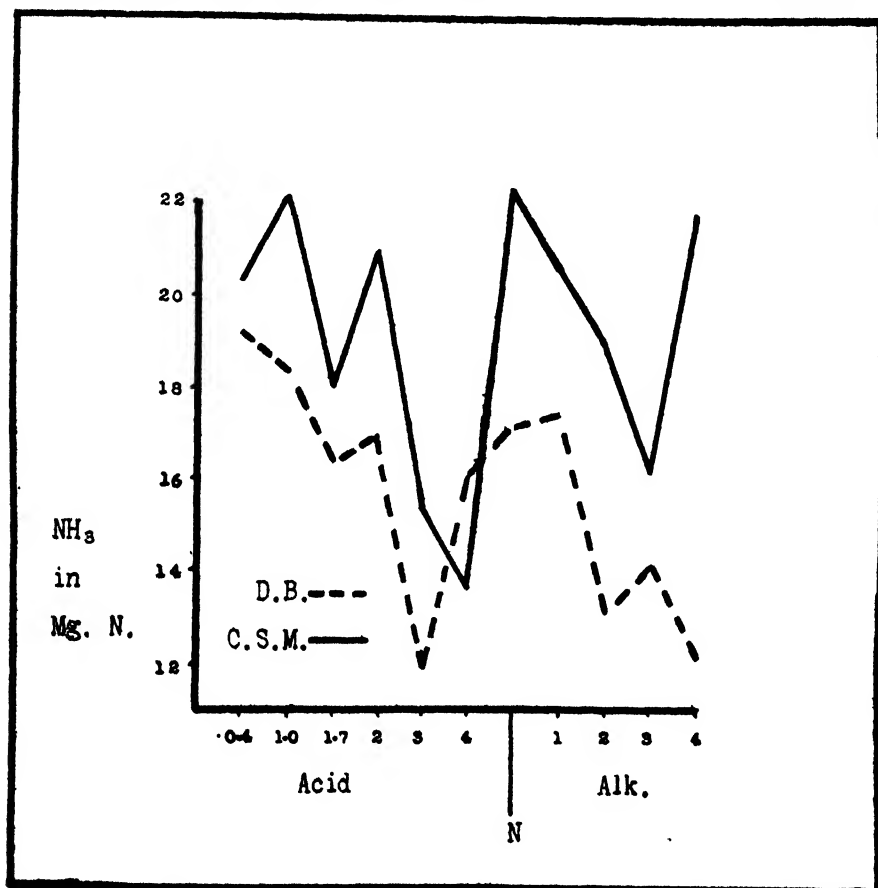


Fig. 1.—The effect of reaction on *Rhizopus nigricans* in Penn clay loam (HCl—NaOH).

ism is directly involved, which is capable of being affected by imperceptible as well as manifest variations, introduces a considerable element of uncertainty even in the chemical phases of such experimentation.

In the alkaline portion of the cottonseed meal series it will be seen that with increasing 1,000-pound applications of CaO per acre (with the exception of the initial one) there is a decrease in ammonia.

Plate IV again shows a correlation of this phenomenon with the growth of mycelium.

Regarding Table I in its entirety, then, it is evident that the reaction of the sandy soil has a profound bearing upon the ammonification of dried blood and cottonseed meal by *Rhizopus nigricans*. In accordance with the general observations heretofore mentioned, a neutral to acid reaction is most favorable to an accumulation of ammonia. However, increasing the acidity beyond 2,000 pounds causes a marked decrease in ammonia. Likewise, increasing the alkalinity causes a gradual decrease in ammonia. Consequently, so far as this organism is concerned, one of its physiological activities, ammonification, is limited by a fairly narrow range of reaction.

TABLE III  
THE EFFECT OF REACTION ON ZYGORRHYNCHUS VUILLEMINII IN  
NORFOLK SANDY LOAM  
(HCl—NaOH)

No.	Organic Matter	Treatment	NH <sub>3</sub> accumulated in			Increase over check Mg. N.
			Mg. N.	Mg. N.	Av. Mg. N	
45-46	155 mg. N. Dried Bl'd	Check .....	3.39	3.55	3.47	.....
47-48	"	Soil L. R. $\approx$ 400 lbs. CaO	10.65	10.65	10.65	7.18
49-50	"	Acid $\approx$ 1000 lbs. CaO....	16.27	16.28	16.28	12.81
51-52	"	Acid $\approx$ 2000 lbs. CaO....	24.42	25.70	25.06	21.59
53-54	"	Acid $\approx$ 3000 lbs. CaO....	21.86	21.72	21.79	18.32
55-56	"	Acid $\approx$ 4000 lbs. CaO....	9.75	10.22	9.98	6.51
57-58	"	Neutral .....	7.81	7.64	7.73	4.26
59-60	"	Alk. $\approx$ 1000 lbs. CaO....	7.45	7.52	7.49	4.02
61-62	"	Alk. $\approx$ 2000 lbs. CaO....	6.81	7.24	7.02	3.55
63-64	"	Alk. $\approx$ 3000 lbs. CaO....	7.51	7.76	7.64	4.17
65-66	"	Alk. $\approx$ 4000 lbs. CaO....	6.17	6.24	6.20	2.73
67-68	155 mg. N. Cottonseed Meal	Check .....	3.69	3.69	3.69	.....
69-70	"	Soil L. R. $\approx$ 400 lbs. CaO	32.23	28.68	30.46	26.77
71-72	"	Acid $\approx$ 1000 lbs. CaO....	29.18	29.39	29.29	25.60
73-74	"	Acid $\approx$ 2000 lbs. CaO....	28.54	28.92	28.73	25.24
75-76	"	Acid $\approx$ 3000 lbs. CaO....	22.93	22.79	22.86	19.17
77-78	"	Acid $\approx$ 4000 lbs. CaO....	17.82	18.46	18.14	14.45
79-80	"	Neutral .....	28.81	29.11	28.96	25.27
81-82	"	Alk. $\approx$ 1000 lbs. CaO....	22.08	20.61	21.35	17.66
83-84	"	Alk. $\approx$ 2000 lbs. CaO....	13.27	14.05	13.66	9.97
85-86	"	Alk. $\approx$ 3000 lbs. CaO....	11.72	10.36	11.04	7.35
87-88	"	Alk. $\approx$ 4000 lbs. CaO....	10.36	11.28	10.82	7.13

Table II shows the effect of reaction on *Rhizopus nigricans* in Penn clay loam, a graphic representation of which appears in figure 1. In this experiment the inoculation consisted of 378,000 spores per 1 c.c. From the results of the dried blood series in Table I it will be seen that an acidity of 400 to 1,000 pounds appears to be most favorable for the accumulation of ammonia. Applications of 1,700, 2,000 and 4,000 pounds gave somewhat lower results. Again, these facts may be more readily explained after the observations on mycelial growth are recorded. Thus

the growth in flasks receiving an acidity of 3,000 pounds was greater than in those having 4,000 pounds. In all of the other instances the

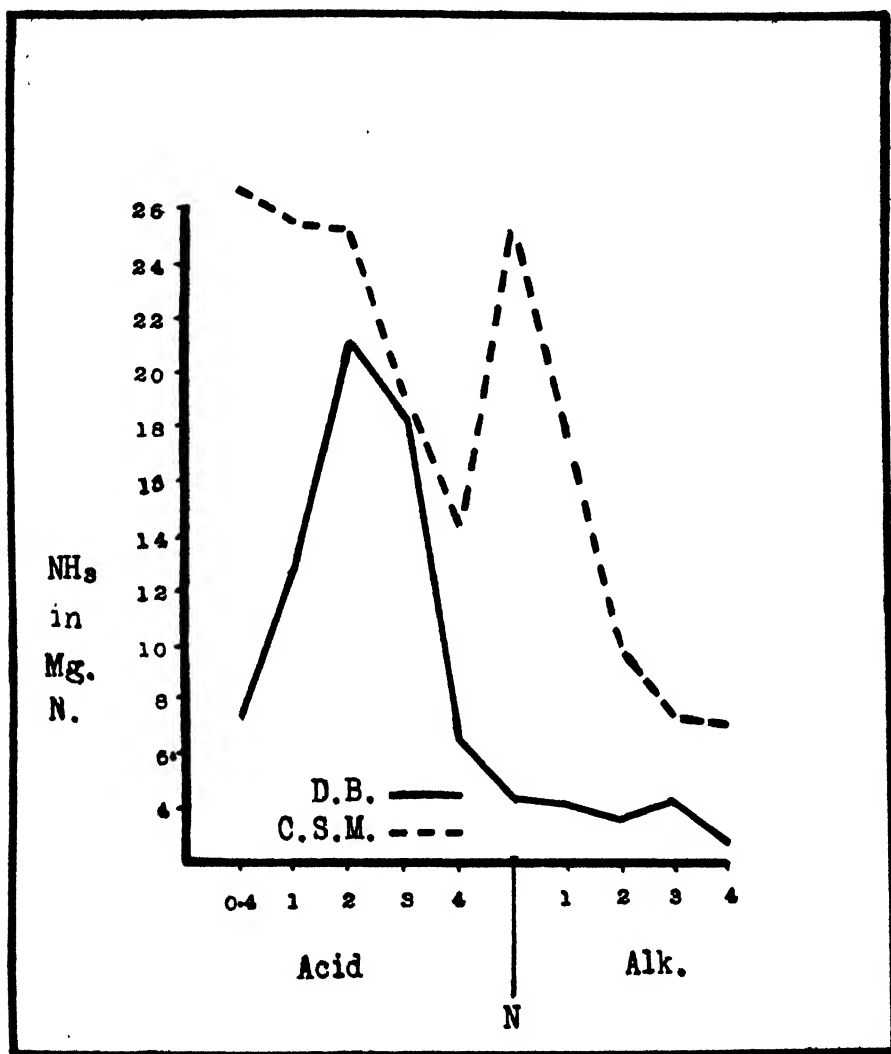


Fig. 2.—The effect of reaction on *Zygorrhynchus Vuilleminii* in Norfolk sandy loam (HCl—NaOH).

amount of mycelial growth could be correlated with ammonia accumulation. Therefore the above-mentioned exceptions would indicate that in those particular cases more ammonia may actually have been produced, but similarly more had been consumed in the development of mycelia. Thus, in effect, it might be argued that a reaction varying from neutral to 1,000 pounds acidity was the most favorable for the accumulation of



ammonia in this soil. Furthermore, there appears to be a tendency towards a decrease in ammonia as the alkalinity is increased. In the cottonseed meal series there is an increase in ammonia with 1,000 pounds acidity compared with 400 pounds. There is a gradual decrease in ammonia as the acidity is increased beyond this point. Again, it is to be noted that the mycelial growth in an acidity of 1,700 pounds was the same as that in 2,000 pounds, and therefore it may be assumed that a reaction between the neutral point and an acidity of 2,000 pounds is most favorable for the ammonification. It is evident that increasing the alkalinity causes a gradual decrease in ammonia (with but one exception).

TABLE IV  
THE EFFECT OF REACTION ON *ZYGORRHYNCUS VUILLEMINII* IN  
PENN CLAY LOAM  
(HCl—NaOH)

No.	Organic Matter	Treatment	NH <sub>3</sub> accumulated in			Increase over check Mg. N.
			Mg. N.	Mg. N.	Av. Mg. N.	
	155 mg. N.					
137-138	Dried Bl'd	Check .....	4.15	3.93	4.04	.....
139-140	"	Soil L. R. $\approx$ 1700 lbs. CaO	10.37	9.50	9.99	5.95
141-142	"	Acid $\approx$ 400 lbs. CaO ...	9.65	9.50	9.58	5.54
143-144	"	Acid $\approx$ 1000 lbs. CaO....	9.50	9.50	9.50	5.46
145-146	"	Acid $\approx$ 2000 lbs. CaO....	12.10	10.08	11.09	7.05
147-148	"	Acid $\approx$ 3000 lbs. CaO....	11.38	10.94	11.16	7.12
149-150	"	Acid $\approx$ 4000 lbs. CaO....	15.12	14.54	14.83	10.79
151-152	"	Neutral .....	10.94	10.22	10.58	6.54
153-154	"	Alk. $\approx$ 1000 lbs. CaO....	9.36	9.94	9.65	5.61
155-156	"	Alk. $\approx$ 2000 lbs. CaO....	9.65	9.22	9.44	5.40
157-158	"	Alk. $\approx$ 3000 lbs. CaO....	9.05	8.64	8.85	4.81
159-160	"	Alk. $\approx$ 4000 lbs. CaO....	9.22	9.05	9.14	5.10
	155 mg. N. Cottonseed					
161-162	Meal	Check .....	3.82	5.17	4.50	.....
163-164	"	Soil L. R. $\approx$ 1700 lbs. CaO	19.15	20.45	19.80	15.30
165-166	"	Acid $\approx$ 400 lbs. CaO....	16.99	16.56	16.78	12.28
167-168	"	Acid $\approx$ 1000 lbs. CaO....	19.01	19.01	19.01	14.51
169-170	"	Acid $\approx$ 2000 lbs. CaO....	21.02	21.89	21.46	16.96
171-172	"	Acid $\approx$ 3000 lbs. CaO....	20.74	19.87	20.31	15.81
173-174	"	Acid $\approx$ 4000 lbs. CaO....	21.02	17.42	19.22	14.72
175-176	"	Neutral .....	18.43	18.29	18.36	13.86
177-178	"	Alk. $\approx$ 1000 lbs. CaO....	14.83	14.83	14.83	10.33
179-180	"	Alk. $\approx$ 2000 lbs. CaO....	14.69	14.40	14.55	10.05
181-182	"	Alk. $\approx$ 3000 lbs. CaO....	11.52	12.24	11.88	7.38
183-184	"	Alk. $\approx$ 4000 lbs. CaO....	9.65	8.64	9.15	4.65

Considering then the data presented, it will be observed that in general with *Rhizopus nigricans*, both in sandy and in clay soils, using both kinds of organic matter, there seems to be a fairly narrow range of tolerance to acidity and alkalinity so far as the maximum ammonia accumulation is concerned.

In Table III are recorded the results dealing with the effect of reaction on *Zygorrhynchus Vuilleminii* in Norfolk sandy loam, which are graphically presented in figure 2. It may be perceived that there is a striking increase in ammonia from the neutral point with an increase in

acidity up to 2,000 pounds. Any increase in acidity beyond this point is marked by a decrease in ammonia. Increasing the alkalinity causes a gradual decrease in ammonia (with but one exception). Considering the cottonseed meal series there is practically an equal amount of ammonia accumulated where the reaction ranges from neutral to 2,000 pounds acid, but an increase in acidity beyond this point is responsible for a decrease in ammonia. As previously noted, an increase in alkalinity is responsible for a gradual decrease in ammonia. Because of the fact that

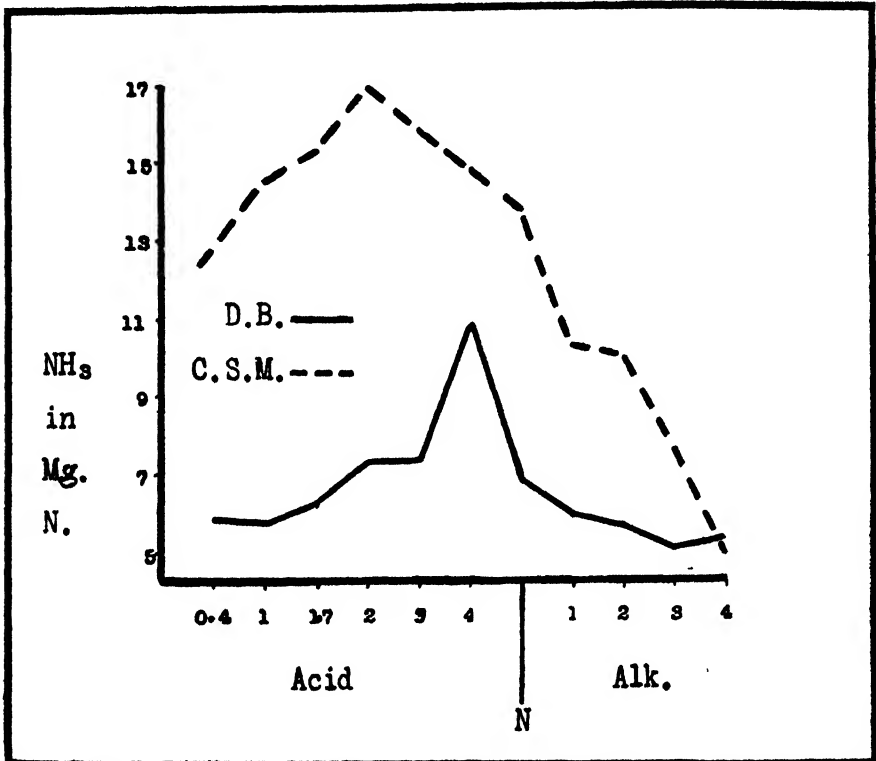


Fig. 3.—The effect of reaction on *Zygorrhynchus Vuilleminii* in Penn clay loam (HCl—NaOH).

*Zygorrhynchus Vuilleminii* does not produce as rank a mycelial growth as *Rhizopus*, it is hardly possible to establish, with any degree of precision, a close correlation between ammonia accumulation and mycelial growth. However, the observations made substantiate the evidence that in general this correlation obtains throughout this work.

In Table IV and figure 3 are set forth the results dealing with the effect of reaction on *Zygorrhynchus Vuilleminii* in Penn clay loam. There were present 55,000 spores per 1 c.c. of inoculum, a fact which accounts for the comparatively small amounts of ammonia accumulated, especially with dried blood, which has been shown, in another connection, to be a

poor source of ammonifiable material for this organism (11). In point of fact, differences manifested by various treatments are in most instances so slight as to permit of no definite conclusions. In the cottonseed meal series, however, it is evident that there is a gradual increase in ammonia with successive increases in acidity up to 2,000 pounds, and thereafter a slight decline may be observed. Increasing the alkalinity is responsible for a gradual decrease in ammonia accumulation.

TABLE V  
THE EFFECT OF REACTION ON *PENICILLIUM* SP. IN NORFOLK SANDY LOAM  
(HCl—NaOH)

No.	Organic Matter	Treatment	NH <sub>3</sub> accumulated in			Increase over check Mg. N.
			Mg. N.	Mg. N.	Av. Mg. N.	
185-186	155 mg. N. Dried Bl'd	Check .....	2.65	2.15	2.40	.....
187-188	"	Acid $\approx$ 1000 lbs. CaO....	20.86	22.12	21.49	19.09
189-190	"	Acid $\approx$ 2000 lbs. CaO....	29.54	30.10	29.82	27.42
191-192	"	Soil L. R. $\approx$ 2300 lbs. CaO	30.94	31.50	31.22	28.82
193-194	"	Acid $\approx$ 3000 lbs. CaO....	28.56	29.26	29.41	27.01
195-196	"	Acid $\approx$ 4000 lbs. CaO....	12.46	12.18	12.32	9.92
197-198	"	Neutral .....	15.96	16.52	16.24	13.84
199-200	"	Alk. $\approx$ 1000 lbs. CaO....	8.82	9.24	9.03	6.63
201-202	"	Alk. $\approx$ 2000 lbs. CaO....	7.98	7.70	7.84	5.44
203-204	"	Alk. $\approx$ 3000 lbs. CaO....	6.02	4.76	5.39	2.99
205-206	"	Alk. $\approx$ 4000 lbs. CaO....	3.78	4.06	3.92	1.52
207-208	155 mg. N. Cottonseed	Check .....	2.71	2.89	2.80	.....
209-210	Meal	Acid $\approx$ 1000 lbs. CaO....	19.88	20.30	20.09	17.29
211-212	"	Acid $\approx$ 2000 lbs. CaO....	26.04	29.26	27.65	24.85
213-214	"	Soil L. R. $\approx$ 2300 lbs. CaO	23.94	22.82	23.38	20.58
215-216	"	Acid $\approx$ 3000 lbs. CaO....	23.10	22.12	22.61	19.81
217-218	"	Acid $\approx$ 4000 lbs. CaO....	11.62	11.34	11.48	8.68
219-220	"	Neutral .....	10.64	11.76	11.20	8.40
221-222	"	Alk. $\approx$ 1000 lbs. CaO....	4.62	3.36	3.99	1.19
223-224	"	Alk. $\approx$ 2000 lbs. CaO....	3.22	3.50	3.36	0.56
225-226	"	Alk. $\approx$ 3000 lbs. CaO....	2.94	2.94	2.94	0.14
227-228	"	Alk. $\approx$ 4000 lbs. CaO....	6.58	6.16	6.37	3.57

Thus considering as a whole the data presented on the effect of reaction on ammonification by *Zygorrhyncus Vuilleminii*, in both sandy and clay soils with the two different sources of organic matter, it appears that the reaction most favorable to maximum ammonia accumulation lies between the rather narrow limits of the neutral point and an acidity of 2,000 pounds. It will be remembered that this coincides with the results obtained with *Rhizopus nigricans* under similar conditions.

In Table V and figure 4 are recorded the results dealing with the effect of reaction on ammonification by *Penicillium* sp. 10 in Norfolk sandy loam. A different sample of this type was used in this experiment with *Penicillium* from that which had been previously employed with *Rhizopus nigricans* and *Zygorrhyncus Vuilleminii*. The sole difference,

however, is to be found in the fact that the present sample had a considerably higher lime requirement, namely, 2,300 pounds CaO per acre. Also, it may be mentioned that a different sample of Penn clay loam was employed, having a lime requirement of 1,100 pounds per acre. In inoculating both sand and clay, there were present 452,000 spores per 1 c.c. of spore-suspension. The flasks were incubated for 7 days, but the ammonification was so slight as to necessitate a repetition of the experiment with a 12-day incubation, the results of which are recorded below.

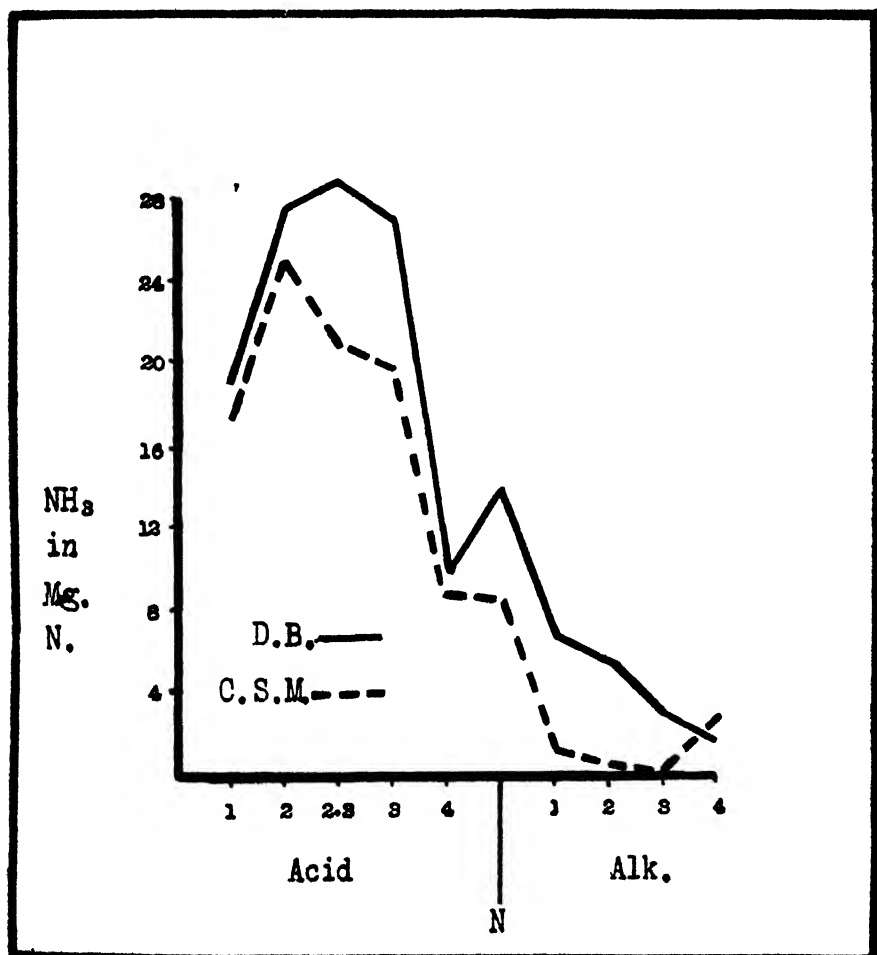


Fig. 4.—The effect of reaction on *Penicillium* sp. in Norfolk sandy loam (HCl—NaOH).

In the dried blood series there is an increase in ammonia from the neutral point to an acidity of 2,300 pounds, above which point a decline sets in. An increase in alkalinity is responsible for a pronounced decrease

in ammonia accumulated. In the cottonseed meal series there is an increase in ammonia with an increase in acidity up to 2,000 pounds, and therefore a decrease in ammonia occurs. Making the soil alkaline beyond the neutral point resulted in a slight accumulation of ammonia which may be regarded as negligible.

TABLE VI  
THE EFFECT OF REACTION ON *PENICILLIUM* SP. IN PENN CLAY LOAM  
(HCl—NaOH)

No.	Organic Matter	Treatment	NH <sub>3</sub> accumulated in			Increase over check Mg. N.
			Mg. N.	Mg. N.	Av. Mg. N.	
229-230	155 mg. N. Dried Bl'd	Check .....	2.91	2.69	2.80	.....
231-232	"	Acid $\approx$ 1000 lbs. CaO....	25.06	26.46	25.76	22.96
233-234	"	Soil L. R. $\approx$ 1100 lbs. CaO	21.70	20.86	21.28	18.48
235-236	"	Acid $\approx$ 2000 lbs. CaO....	29.68	25.48	27.58	24.78
237-238	"	Acid $\approx$ 2300 lbs. CaO....	32.20	29.26	30.73	27.93
239-240	"	Acid $\approx$ 3000 lbs. CaO....	23.24	23.94	23.59	20.79
241-242	"	Acid $\approx$ 4000 lbs. CaO....	23.94	22.68	23.31	20.51
243-244	"	Neutral .....	20.44	22.54	21.49	18.69
245-246	"	Alk. $\approx$ 1000 lbs. CaO....	21.98	22.26	22.12	19.32
247-248	"	Alk. $\approx$ 2000 lbs. CaO....	16.66	21.42	19.04	16.24
249-250	"	Alk. $\approx$ 3000 lbs. CaO....	18.62	18.62	18.62	15.82
251-252	"	Alk. $\approx$ 4000 lbs. CaO....	14.84	13.44	14.14	11.34
253-254	155 mg. N. Cottonseed Meal	Check .....	3.93	3.87	3.90	.....
255-256	"	Acid $\approx$ 1000 lbs. CaO....	15.26	16.94	16.10	12.20
257-258	"	Soil L. R. $\approx$ 1100 lbs. CaO	19.88	17.64	18.76	14.86
259-260	"	Acid $\approx$ 2000 lbs. CaO....	19.18	20.74	19.96	16.06
261-262	"	Acid $\approx$ 2300 lbs. CaO....	17.08	17.36	17.22	13.32
263-264	"	Acid $\approx$ 3000 lbs. CaO....	15.26	15.68	15.47	11.57
265-266	"	Acid $\approx$ 4000 lbs. CaO....	12.18	11.06	11.62	7.72
267-268	"	Neutral .....	17.36	16.38	16.87	12.97
269-270	"	Alk. $\approx$ 1000 lbs. CaO....	13.72	13.16	13.44	9.54
271-272	"	Alk. $\approx$ 2000 lbs. CaO....	9.10	7.70	8.40	4.50
273-274	"	Alk. $\approx$ 3000 lbs. CaO....	4.90	7.28	6.09	2.19
275-276	"	Alk. $\approx$ 4000 lbs. CaO....	5.04	5.88	5.46	1.56

In Table VI and figure 5 which show the effect of reaction on ammonification by *Penicillium* sp. 10 in Penn clay loam, it will be seen that there is an increase in ammonia with an increase in acidity up to 2,300 pounds, following which a decrease ensues (with but one exception). Increasing the alkalinity causes a corresponding decrease in ammonia. In the cottonseed meal series there is an increase in acidity up to 2,000 pounds, followed by a decrease in ammonia beyond this point. Again, it is obvious that an increase in alkalinity is responsible for a marked decrease in ammonia.

Therefore in this experiment where a sandy soil of high lime requirement and a clay soil of lower lime requirement than that previously used were employed, with dried blood as the source of organic matter, the maximum ammonia accumulation occurred with a reaction varying from the neutral point to 2,300 pounds acidity. Where cottonseed meal was

used, the maximum ammonia accumulation took place at 2,000 pounds acid. It is possible that the reason for this difference is that cottonseed meal might induce a more acid condition in the soil than dried blood in this period of time. Therefore a slightly smaller addition of acidity, i. e., 2,000 pounds, to a soil receiving cottonseed meal would be as efficient as the application of an acidity of 2,300 pounds where dried blood was used, so far as maximum ammonia accumulation with this fungus was concerned. In all cases an increase in alkalinity was responsible for a decrease in ammonia accumulation.

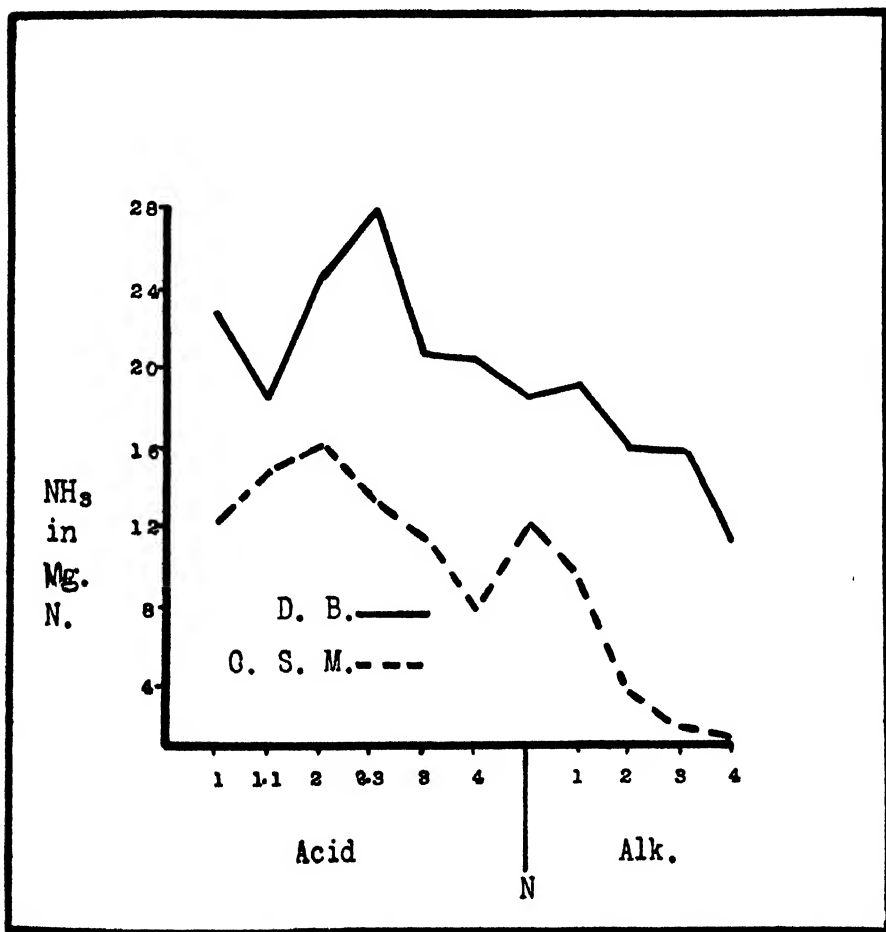


Fig. 5.—The effect of reaction on *Penicillium* sp. in Penn clay loam (HCl—NaOH).

Recapitulating the salient features brought out in the preceding discussion, the following points may be established with due regard for the limitations involved in the experiment.

1. Using normal solutions of HCl and NaOH to alter the soil reaction, it was found that the latter had a profound bearing upon the ammonification of organic nitrogenous materials by *Rhizopus nigricans*, *Zygorrhynchus Vuilleminii*, and *Penicillium* sp. 10, all of which were influenced in the same manner.

2. The effect of soil reaction upon the ammonification of dried blood by these fungi was practically the same as that of cottonseed meal.

3. The effect of soil reaction on the ammonification of these materials by the fungi employed was practically the same in sandy or clay loam of high or of low lime requirement.

4. The maximum ammonia accumulated by these organisms using either of the soils with either of the organic materials occurred when the reaction of the soil lay between the neutral point and an acidity equivalent to 2,000 pounds CaO per acre.

5. Increasing the acidity beyond this point, or increasing alkalinity beyond the neutral point usually was responsible for a corresponding decrease in ammonia accumulated.

6. In general, whenever such observation was possible, it was found that mycelial growth could be correlated with ammonia accumulation.

## II

### *The Effect of Soil Reaction on Ammonification by Soil Fungi when the Reaction has been Altered by Additions of $\text{CaCO}_3$ and $\text{H}_2\text{SO}_4$ .*

Having established the range of soil reaction which was most advantageous to a maximum accumulation of ammonia by these organisms, it was deemed advisable to make the inquiry somewhat more practical in its bearing by using  $\text{CaCO}_3$  instead of NaOH and substituting  $\text{H}_2\text{SO}_4$  for the HCl previously employed. It is obvious that in actual field practice soil acidity is corrected by applications of lime. Since caustic lime has an antiseptic property, it was considered more desirable to use calcium carbonate. Sulfuric acid was chosen in preference to other acids because when used in conjunction with  $\text{CaCO}_3$  it made possible the use of a neutral compound namely  $\text{CaSO}_4$  which contains the most important radicals of the two materials employed. Furthermore sulfur is not used by fungi as a nutrient to as great an extent as is carbon which would be present in any organic acid that might be worthy of consideration.

Stevens (21) states that *Penicillium* spores grow in N/50  $\text{H}_2\text{SO}_4$  and Traaen (23) maintains that  $\text{H}_2\text{SO}_4$  is not as toxic to fungi as HCl. Planchon (17) likewise found that  $\text{H}_2\text{SO}_4$  was advantageous to the development of molds.

The question of whether or not calcium is an essential element of food for fungi or can function as such if replacing magnesium is at present a somewhat disputed point. Sauton (20) states that Ca cannot replace Mg as an essential element and in fact depresses fungous growth.

Robert (19) found that there was a slight increase in the weight of fungi proportional to the amount of Ca added, provided the latter were small. Winogradsky (25) states that Ca is not an essential element and cannot replace Mg. Buromsky (2) quotes the work of Molisch and others to show that Ca cannot replace Mg but increases the yield of fungous growth somewhat. According to Buromsky, Ca cannot serve as a

TABLE VII  
THE EFFECT OF REACTION ON *PENICILLIUM* SP. IN NORFOLK SANDY LOAM  
( $\text{H}_2\text{SO}_4\text{--CaCO}_3$ )

No.	Organic Matter	Treatment	$\text{NH}_3$ accumulated in			Increase over check Mg. N.
			Mg. N.	Mg. N.	Av. Mg. N.	
	155 mg. N. Dried Bl'd	Check .....	2.53	2.27	2.40	.....
1-2	"	Acid $\approx$ 1000 lbs. CaO....	22.26	23.52	22.89	20.49
3-4	"	Acid $\approx$ 2000 lbs. CaO...	27.44	29.12	28.28	25.88
5-6	"	Soil L. R $\approx$ 2300 lbs. CaO	29.50	30.00	29.75	27.35
7-8	"	Acid $\approx$ 3000 lbs. CaO....	26.49	29.18	27.89	25.49
9-10	"	Acid $\approx$ 4000 lbs. CaO...	25.70	25.14	25.42	23.02
11-12	"	Neutral .....	15.26	13.44	14.35	11.95
13-14	"	Alk $\approx$ 1000 lbs. CaO....	10.08	10.15	10.15	7.75
15-16	"	Alk. $\approx$ 2000 lbs. CaO...	8.68	7.98	8.33	5.93
17-18	"	Alk $\approx$ 3000 lbs. CaO...	8.40	7.70	8.05	5.65
19-20	"	Alk. $\approx$ 4000 lbs. CaO...	6.72	7.56	7.14	4.74
21-22	"	Alk. $\approx$ 10,000 lbs. CaO....	11.60	11.35	11.48	9.08
23-24	"	Alk. $\approx$ 20,000 lbs. CaO...	9.60	9.82	9.71	7.31
25-26	"	Alk. $\approx$ 30,000 lbs. CaO...	10.00	9.60	9.80	7.40
27-28	"	Alk. $\approx$ 40,000 lbs. CaO...	10.00	9.85	9.93	7.53
29-30	"	Alk. $\approx$ 50,000 lbs. CaO...	10.04	9.50	9.77	7.37

nutrient for fungi and depresses the yield of *Aspergillus niger*. Butkevitch (3) found that  $\text{CaCO}_3$  in amounts of 2 and 10 gm. in 50 c.c. of nutrient solution containing 4 per cent peptone, 0.2 per cent sugar and 0.2 per cent  $\text{NaCl}$  depressed the production of ammonia to one-fourth of the quantity produced in the absence of  $\text{CaCO}_3$ . A review of the literature then indicates that Ca is not an essential nutrient for fungi and that in small amounts it may act as a stimulant, while in larger amounts it may actually depress the growth of these organisms.

In the following experiments the Norfolk sandy loam used had a lime requirement of 2,300 pounds CaO per acre on the basis of 3,000,000 pounds of soil per surface 6 2/3 inches and the Penn clay loam had a lime requirement of 1,100 pounds per acre on the basis of 2,700,000 pounds of soil per surface 6 2/3 inches. The method of procedure was identical with that previously outlined. A normal solution of  $\text{H}_2\text{SO}_4$  and  $\text{CaCO}_3$  (c. p.) were employed to alter the reaction of the soil. As before, in these experiments the same gradations obtained, except that in the alkalinity series where the applications were increased as high as 50,000 pounds CaO per acre, or practically 2 per cent, to guard against the possible influence of either the Ca or  $\text{SO}_4$  radicals,  $\text{CaSO}_4$  was applied at the outset to the flasks in quantities equivalent to the highest amounts of



those radicals used. Later this practice was considered superfluous and was discarded.

Considering the effect of reaction on *Penicillium* sp. 10 (using 132,000 spores per 1 c.c. of inoculum) in Norfolk sandy loam as shown in Table VII and figure 6 in the dried blood series, it is evident that there is a pronounced increase in ammonia accumulation as the acidity is in-

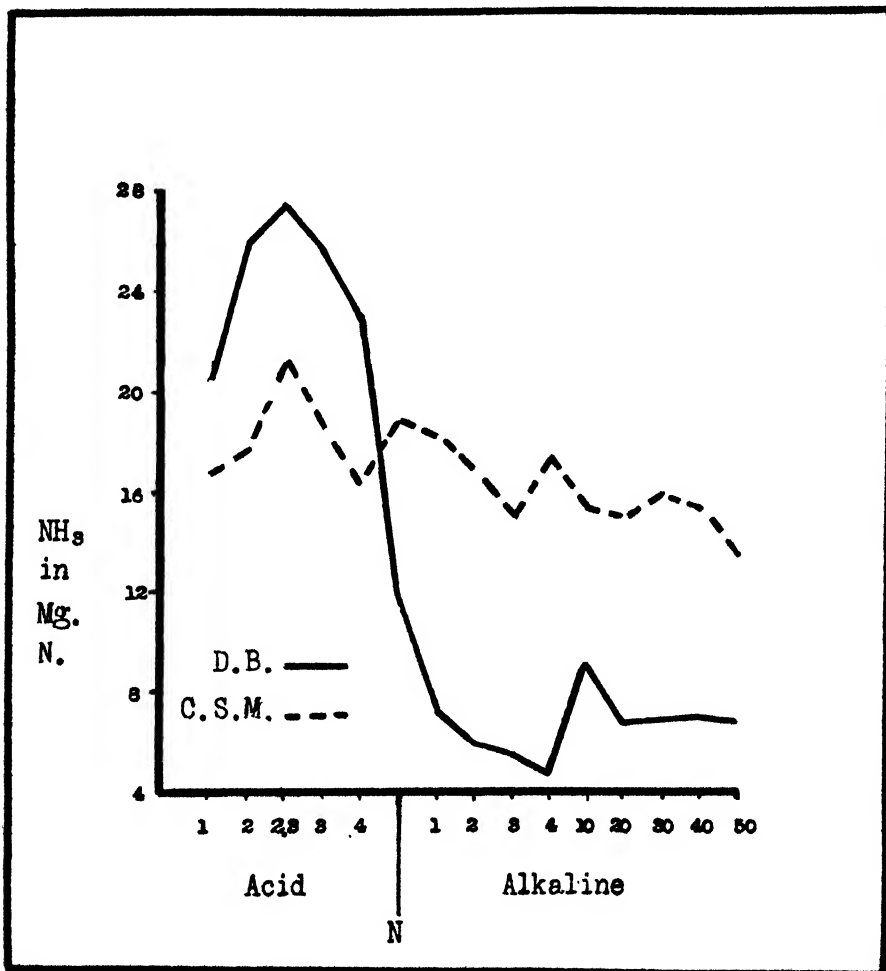


Fig. 6.—The effect of reaction on *Penicillium* sp. in Norfolk sandy loam ( $\text{H}_2\text{SO}_4$ — $\text{CaCO}_3$ ).

creased from the neutral point to an acidity of 2,300 pounds. Thereafter, there is a decrease in ammonia. With an increase in alkalinity beyond the neutral point there is a decrease in ammonia, although this is not proportional to the increase in application of  $\text{CaCO}_3$  above 4,000 pounds. In point of fact the addition of 10,000 to 50,000 lbs  $\text{CaO}$  per acre yielded a greater amount of ammonia than the smaller applications.

Owing to the nature of the growth of this organism in soil it does not readily permit of the correlation of mycelial growth with ammonia accumulation. Thus speculation might be advanced to the effect that the explanation of the above phenomenon depended on the greater production of ammonia, but likewise greater consumption in the process of growth.

TABLE VIII  
THE EFFECT OF REACTION ON *PENICILLIUM* SP. IN NORFOLK SANDY LOAM  
( $H_2SO_4-CaCO_3$ )

No.	Organic Matter	Treatment	NH <sub>3</sub> accumulated in			Increase over check Mg. N.
			Mg. N.	Mg. N.	Av. Mg. N.	
	155 mg. N. Cottonseed Meal	Check .....	2 91	2.69	2.80	...
31-32	"	Acid $\approx$ 1000 lbs. CaO....	20.44	18.62	19.53	16.73
33-34	"	Acid $\approx$ 2000 lbs. CaO. .	19 74	21 28	20.51	17.71
35-36	"	Soil L. R. $\approx$ 2300 lbs. CaO	24 75	22 85	23.80	21 00
37-38	"	Acid $\approx$ 3000 lbs. CaO....	19.43	23 10	21 27	18 47
39-40	"	Acid $\approx$ 4000 lbs. CaO....	19 88	18 15	19.02	16.22
41-42	"	Neutral .....	21 28	21.98	21 63	18 83
43-44	"	Alk. $\approx$ 1000 lbs. CaO... .	21.84	20 30	21.07	18.27
45-46	"	Alk. $\approx$ 2000 lbs. CaO... .	21 56	18.90	20 23	17 43
47-48	"	Alk. $\approx$ 3000 lbs CaO ...	17 22	18 62	17.92	15.71
49-50	"	Alk. $\approx$ 4000 lbs. CaO.....	20 72	20 30	20.51	17.12
51-52	"	Alk. $\approx$ 10,000 lbs. CaO... .	18.65	17.80	18.23	15 43
53-54	"	Alk. $\approx$ 20,000 lbs CaO .	17.89	17.85	17 87	15.07
55-56	"	Alk. $\approx$ 30,000 lbs. CaO ..	18 80	18.70	18 75	15 95
57 58	"	Alk. $\approx$ 40,000 lbs CaO... .	18.30	17 85	18.08	15.28
59 60	"	Alk. $\approx$ 50,000 lbs. CaO)	17 18	15.41	16 25	13 45

In the cottonseed meal series, the results of which are given in Table VIII and figure 6, it is again apparent that with an increase in acidity of 1,000 to 2,300 pounds there is a gradual increase in ammonia accumulation. Beyond this point there is perceptible decline. While an increase in alkalinity from 1,000 to 4,000 pounds (with one exception) causes a decrease in ammonia, at the latter point practically a constant ensues.

In Table IX and figure 7 is shown the effect of reaction on ammonification by *Penicillium* sp. 10. in Penn clay loam using dried blood as a source of organic matter. With an increase in acidity from 1,000 to 2,000 pounds there is an increase in ammonia. But a further increase to 3,000 pounds does not cause any decline such as takes place when the acidity is increased to 4,000 pounds. Increasing the alkalinity from 1,000 to 4,000 pounds causes a corresponding decrease in ammonia. Further applications have no influence since practically a constant is maintained. These results are found to be in agreement with those obtained by McLean and Wilson (14) with another species of *Penicillium* in the same soil.

In the cottonseed meal series, the results of which appear in Table X and figure 7, the maximum ammonia accumulation occurs between the neutral point and an acidity of 2,000 pounds. Above this point there is a gradual decline in ammonia accumulation. Increasing the alkalinity

causes a corresponding decrease in ammonia to 3,000 pounds, following which practically a constant is maintained.

Thus considering as a whole the data presented on the effect of reaction on ammonification by *Penicillium* in sandy and clay soils using both kinds of organic matter, it is evident that the maximum ammonia accumulation occurs with a reaction between the neutral point and an acidity of 2,300 pounds. Increasing the acidity beyond this point causes a decrease in ammonia accumulation. Again, increasing the alkalinity up to 4,000 pounds causes a decrease in ammonia. Applications beyond this point do not result in any corresponding change in ammonia, since a constant ensues which is usually about the same in quantity as that with 2,000 to 3,000 pounds. The only explanation that suggests itself is that

TABLE IX  
THE EFFECT OF REACTION ON *PENICILLIUM* SP. IN PENN CLAY LOAM  
( $H_2SO_4$ — $CaCO_3$ )

No.	Organic Matter	Treatment	NH <sub>3</sub> accumulated in			Increase over check Mg. N.
			Mg. N.	Mg. N.	Av. Mg. N.	
	155 mg. N. Dried Bld.	Check .....	2.73	2.87	2.80	.....
61-62	"	Acid $\approx$ 1000 lbs. $CaO$ ...	18.90	20.02	19.46	16.66
63-64	"	Soil L. R. $\approx$ 1100 lbs. $CaO$	21.50	21.15	21.33	18.53
65-66	"	Acid $\approx$ 2000 lbs $CaO$ ...	23.10	22.70	22.90	20.10
67-68	"	Acid $\approx$ 2300 lbs. $CaO$ ...	23.12	22.70	22.91	20.11
69-70	"	Acid $\approx$ 3000 lbs. $CaO$ ...	22.85	22.98	22.92	20.12
71-72	"	Acid $\approx$ 4000 lbs. $CaO$ ...	22.40	21.32	21.86	19.06
73-74	"	Neutral .....	18.70	20.60	19.65	16.85
75-76	"	Alk. $\approx$ 1000 lbs. $CaO$ ...	10.50	10.78	10.64	7.84
77-78	"	Alk. $\approx$ 2000 lbs. $CaO$ ...	8.26	8.54	8.40	5.60
79-80	"	Alk. $\approx$ 3000 lbs. $CaO$ ...	6.30	6.30	6.30	3.50
81-82	"	Alk. $\approx$ 4000 lbs. $CaO$ ...	5.46	5.60	5.53	2.73
83-84	"	Alk. $\approx$ 10,000 lbs. $CaO$ ...	8.47	9.00	8.74	5.94
85-86	"	Alk. $\approx$ 20,000 lbs. $CaO$ ...	7.70	7.77	7.74	4.94
87-88	"	Alk. $\approx$ 30,000 lbs. $CaO$ ...	8.28	8.10	8.19	5.39
89-90	"	Alk. $\approx$ 40,000 lbs. $CaO$ ...	7.78	7.84	7.81	5.01
91-92	"	Alk. $\approx$ 50,000 lbs. $CaO$ ...	7.65	7.75	7.70	4.90

possibly the addition of such large quantities of  $CaCO_3$ , may improve the texture of the soil to such an extent as to make the increased oxygen supply an advantageous factor in ammonia accumulation.

Considering the effect of reaction on ammonification by *Zygorrhynchus Vuilleminii* (using 32,000 spores per 1 c.c. of inoculum) in Norfolk sandy loam where dried blood was used, as shown in Table XI and figure 8, it is evident that with an increased acidity from the neutral point to 2,000 pounds, there is a corresponding increase in ammonia. Above the latter point, a further increase in acidity causes a decrease in ammonia. An increase in alkalinity beyond the neutral point allows of a negligible amount of ammonia accumulation.

In the cottonseed meal series as shown in Table XII and figure 8, an increase in acidity from the neutral point to 2,000 pounds causes a cor-

responding increase in ammonia accumulation. Beyond the latter point there is a decline in ammonia. In all probability the fact that more ammonia was accumulated in the presence of an acidity of 4,000 than was the case with 3,000 pounds is due to a greater consumption of ammonia in the latter case. With increasing alkalinity there appears to be a tendency towards a diminution of ammonia, but the variations permit of no definite conclusion.

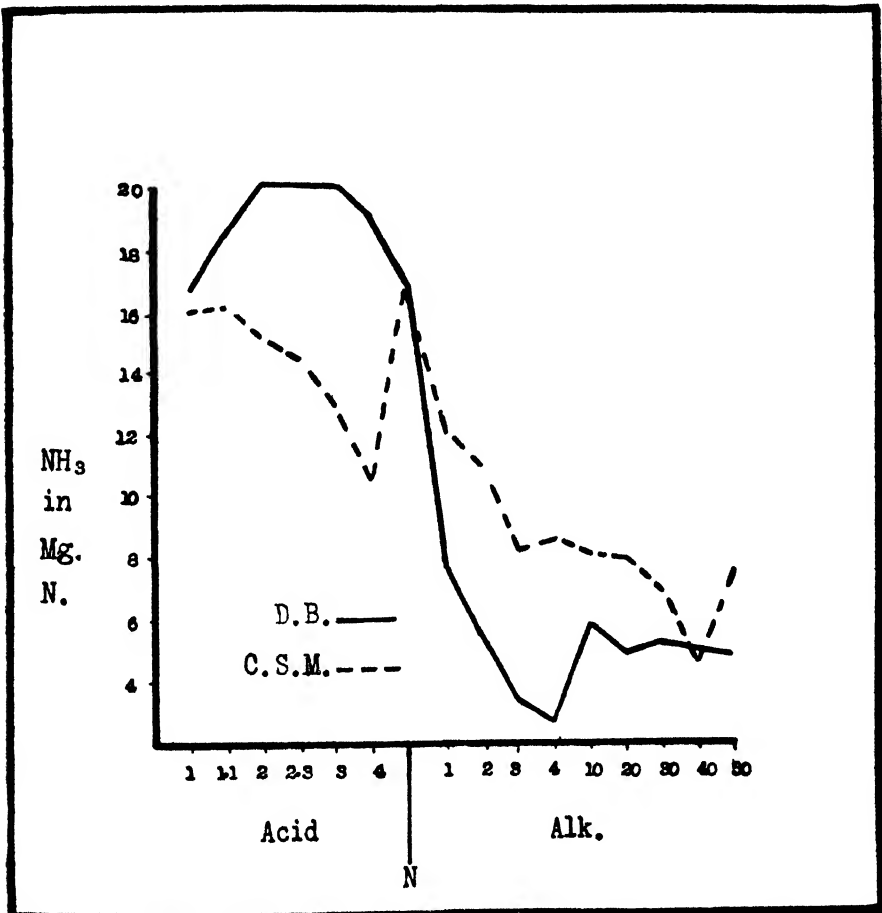


Fig. 7.—The effect of reaction on *Penicillium* sp. in Penn clay loam ( $\text{H}_2\text{SO}_4$ — $\text{CaCO}_3$ ).

The effect of reaction on ammonification by *Zygorrhynchus Vuilleminii* in Penn clay loam using dried blood are given in Table XIII and figure 9. It is evident that there is an increase in ammonia with an increase in acidity from the neutral point to 2,300 pounds. There is a decrease where an acidity of 3,000 pounds obtains, but this is not continued in the case of 4,000 pounds. It is difficult to account for this singular exception. In-

TABLE X  
THE EFFECT OF REACTION ON *PENICILLIUM* SP. IN PENN CLAY LOAM  
( $H_2SO_4-CaCO_3$ )

No.	Organic Matter	Treatment	NH <sub>3</sub> accumulated in			Increase over check Mg. N.
			Mg. N.	Mg. N.	Av. Mg. N.	
	155 mg. N. Cottonseed Meal	Check .....	3.32	3.08	3.20	.....
93-94	"	Acid $\approx$ 1000 lbs. CaO....	19.18	19.32	19.25	16.05
95-96	"	Soil L. R. $\approx$ 1100 lbs. CaO	19.40	19.40	19.40	16.20
97-98	"	Acid $\approx$ 2000 lbs. CaO....	18.46	18.93	18.70	15.50
99-100	"	Acid $\approx$ 2300 lbs. CaO....	17.80	17.76	17.78	14.58
101-102	"	Acid $\approx$ 3000 lbs. CaO....	16.65	15.80	16.23	13.03
103-104	"	Acid $\approx$ 4000 lbs. CaO....	14.35	13.50	13.93	10.73
105-106	"	Neutral .....	19.72	20.55	20.14	16.94
107-108	"	Alk. $\approx$ 1000 lbs. CaO....	15.40	15.12	15.26	12.06
109-110	"	Alk. $\approx$ 2000 lbs. CaO....	14.70	14.00	14.35	11.15
111-112	"	Alk. $\approx$ 3000 lbs. CaO....	11.48	11.48	11.48	8.28
113-114	"	Alk. $\approx$ 4000 lbs. CaO....	11.20	13.44	12.32	9.12
115-116	"	Alk. $\approx$ 10,000 lbs. CaO...	11.47	11.40	11.44	8.24
117-118	"	Alk. $\approx$ 20,000 lbs. CaO...	11.10	11.40	11.25	8.05
119-120	"	Alk. $\approx$ 30,000 lbs. CaO...	10.80	10.20	10.50	7.30
121-122	"	Alk. $\approx$ 40,000 lbs. CaO...	7.98	Lost	7.98	4.78
123-124	"	Alk. $\approx$ 50,000 lbs. CaO...	11.60	10.05	10.83	7.63

TABLE XI  
THE EFFECT OF REACTION ON *ZYGORRHYNCHUS VULLEMINI* IN  
NORFOLK SANDY LOAM  
( $H_2SO_4-CaCO_3$ )

No.	Organic Matter	Treatment	NH <sub>3</sub> accumulated in			Increase over check Mg. N.
			Mg. N.	Mg. N.	Av. Mg. N.	
	155 mg. N. Dried Bl'd	Check .....	2.53	2.27	2.40	.....
125-126	"	Acid $\approx$ 1000 lbs. CaO....	5.39	5.41	5.40	3.00
127-128	"	Acid $\approx$ 2000 lbs. CaO....	10.74	10.68	10.71	8.31
129-130	"	Soil L. R. $\approx$ 2300 lbs. CaO	7.28	9.10	8.19	5.79
131-132	"	Acid $\approx$ 3000 lbs. CaO....	3.95	4.55	4.25	1.85
133-134	"	Acid $\approx$ 4000 lbs. CaO....	3.78	6.72	5.25	2.85
135-136	"	Neutral .....	2.95	3.00	2.98	0.58
137-138	"	Alk. $\approx$ 1000 lbs. CaO....	2.80	2.57	2.69	0.29
139-140	"	Alk. $\approx$ 2000 lbs. CaO....	3.39	3.30	3.35	0.95
141-142	"	Alk. $\approx$ 3000 lbs. CaO....	2.80	2.81	2.81	0.41
143-144	"	Alk. $\approx$ 4000 lbs. CaO....	2.75	2.50	2.63	0.23
145-146	"	Alk. $\approx$ 10,000 lbs. CaO...	4.34	4.34	4.34	1.94
147-148	"	Alk. $\approx$ 20,000 lbs. CaO...	3.29	3.92	3.61	1.21
149-150	"	Alk. $\approx$ 30,000 lbs. CaO...	3.78	3.71	3.75	1.35
151-152	"	Alk. $\approx$ 40,000 lbs. CaO...	3.92	3.78	3.85	1.45
153-154	"	Alk. $\approx$ 50,000 lbs. CaO...	3.50	3.06	3.28	0.88

creasing the alkalinity up to 4,000 pounds reduced the ammonia accumulation to such a degree as to make differences in treatment insignificant. However, the phenomenon previously noted, namely the slight increase in ammonia with large applications of  $CaCO_3$  is again apparent.

In the cottonseed meal series shown in Table XIV and figure 9, the variations in that part of the experiment dealing with acidity do not permit of any definite conclusion, other than that an increase in acidity beyond 3,000 pounds causes a decreased ammonia accumulation. With increased alkalinity up to 10,000 pounds there is a gradual decrease in ammonia. Additions of  $\text{CaCO}_3$  beyond this point produce no differences worthy of note.

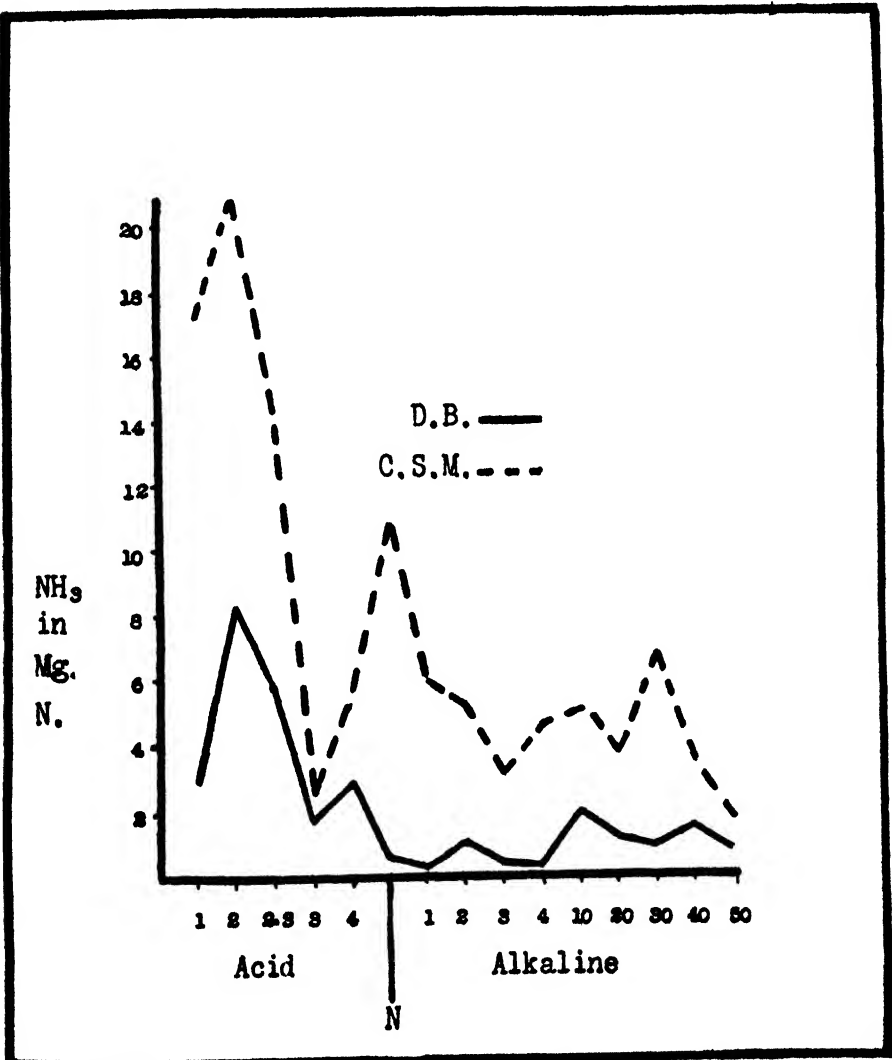


Fig. 8.—The effect of reaction on *Zygorrhynchus Vuilleminii* in Norfolk sandy loam ( $\text{H}_2\text{SO}_4\text{—CaCO}_3$ ).

In general, while the results concerning the effect of reaction on ammonification indicate clearly that in sandy soil with both kinds of or-

TABLE XII  
THE EFFECT OF REACTION ON ZYGORRHYNUS VUILLEMINII IN  
NORFOLK SANDY LOAM  
( $H_2SO_4$ — $CaCO_3$ )

No.	Organic Matter	Treatment	NH <sub>3</sub> accumulated in			Increase over check Mg. N.
			Mg. N.	Mg. N.	Av. Mg. N.	
	155 mg. N. Cottonseed Meal	Check .....	2.91	2.69	2.80	.....
155-156	"	Acid $\approx$ 1000 lbs. $CaO$ ....	19.80	20.20	20.00	17.20
157-158	"	Acid $\approx$ 2000 lbs. $CaO$ ....	23.80	23.72	23.72	20.92
159-160	"	Soil L. R. $\approx$ 2300 lbs. $CaO$	17.70	18.22	17.96	14.16
161-162	"	Acid $\approx$ 3000 lbs. $CaO$ ....	4.90	5.88	5.39	2.59
163-164	"	Acid $\approx$ 4000 lbs. $CaO$ ....	7.49	9.10	8.30	5.50
165-166	"	Neutral .....	13.70	13.57	13.64	10.84
167-168	"	Alk. $\approx$ 1000 lbs. $CaO$ ....	8.98	8.80	8.89	6.09
169-170	"	Alk. $\approx$ 2000 lbs. $CaO$ ....	8.40	7.90	8.15	5.35
171-172	"	Alk. $\approx$ 3000 lbs. $CaO$ ....	6.40	6.40	6.40	3.60
173-174	"	Alk. $\approx$ 4000 lbs. $CaO$ ....	7.90	7.07	7.49	4.69
175-176	"	Alk. $\approx$ 10,000 lbs. $CaO$ ...	9.16	6.78	7.97	5.17
177-178	"	Alk. $\approx$ 20,000 lbs. $CaO$ ...	6.29	7.06	6.67	3.87
179-180	"	Alk. $\approx$ 30,000 lbs. $CaO$ ...	9.58	9.58	9.58	6.78
181-182	"	Alk. $\approx$ 40,000 lbs. $CaO$ ...	6.78	5.80	6.29	3.49
183-184	"	Alk. $\approx$ 50,000 lbs. $CaO$ ...	5.97	3.31	4.64	1.84

TABLE XIII  
THE EFFECT OF REACTION ON ZYGORRHYNUS VUILLEMINII IN  
PENN CLAY LOAM  
( $H_2SO_4$ — $CaCO_3$ )

No.	Organic Matter	Treatment	NH <sub>3</sub> accumulated in			Increase over check Mg. N.
			Mg. N.	Mg. N.	Av. Mg. N.	
	155 mg. N. Dried Bl'd	Check .....	2.73	2.87	2.80	.....
185-186	"	Acid $\approx$ 1000 lbs. $CaO$ ....	6.80	6.85	6.83	4.03
187-188	"	Soil L. R. $\approx$ 1100 lbs. $CaO$	6.83	7.42	7.13	4.33
189-190	"	Acid $\approx$ 2000 lbs. $CaO$ ....	9.38	9.24	9.31	6.51
191-192	"	Acid $\approx$ 2300 lbs. $CaO$ ....	8.89	10.64	9.77	6.97
193-194	"	Acid $\approx$ 3000 lbs. $CaO$ ....	9.66	7.91	8.79	5.99
195-196	"	Acid $\approx$ 4000 lbs. $CaO$ ....	10.08	10.50	10.29	7.49
197-198	"	Neutral .....	5.88	6.30	6.09	3.29
199-200	"	Alk. $\approx$ 1000 lbs. $CaO$ ....	4.00	4.00	4.00	1.20
201-202	"	Alk. $\approx$ 2000 lbs. $CaO$ ....	3.99	4.24	4.12	1.32
203-204	"	Alk. $\approx$ 3000 lbs. $CaO$ ....	3.45	3.36	3.41	0.61
205-206	"	Alk. $\approx$ 4000 lbs. $CaO$ ....	3.50	3.60	3.55	0.75
207-208	"	Alk. $\approx$ 10,000 lbs. $CaO$ ...	4.90	4.90	4.90	2.10
209-210	"	Alk. $\approx$ 20,000 lbs. $CaO$ ...	6.09	4.20	5.15	2.35
211-212	"	Alk. $\approx$ 30,000 lbs. $CaO$ ...	5.60	5.04	5.32	2.52
213-214	"	Alk. $\approx$ 40,000 lbs. $CaO$ ...	4.34	4.34	4.34	1.54
215-216	"	Alk. $\approx$ 50,000 lbs. $CaO$ ...	4.62	4.48	4.55	1.75

ganic matter, an increase in acidity from the neutral point to 2,000 pounds causes a corresponding increase in ammonia accumulation. Above the latter point there appears to be a decrease in ammonia. In clay soil a slightly greater acidity, 2,300 to 3,000 pounds produces the maximum ammonia accumulation. It is possible to suppose that the reason

for the fact that a greater acidity is necessary for maximum ammonia accumulation in clay than in sandy soil, lies in the fact that the distribution of any acid would be more thorough in the case of the sandy soil and consequently a smaller amount would be required. In both soils a further increase in acidity was responsible for a decrease in ammonia. An increase in alkalinity from 1,000 to 4,000 pounds was responsible, generally speaking, for a diminution of ammonia; while applications from 10,000 to 50,000 pounds of CaO per acre yielded approximately a constant quantity of ammonia.

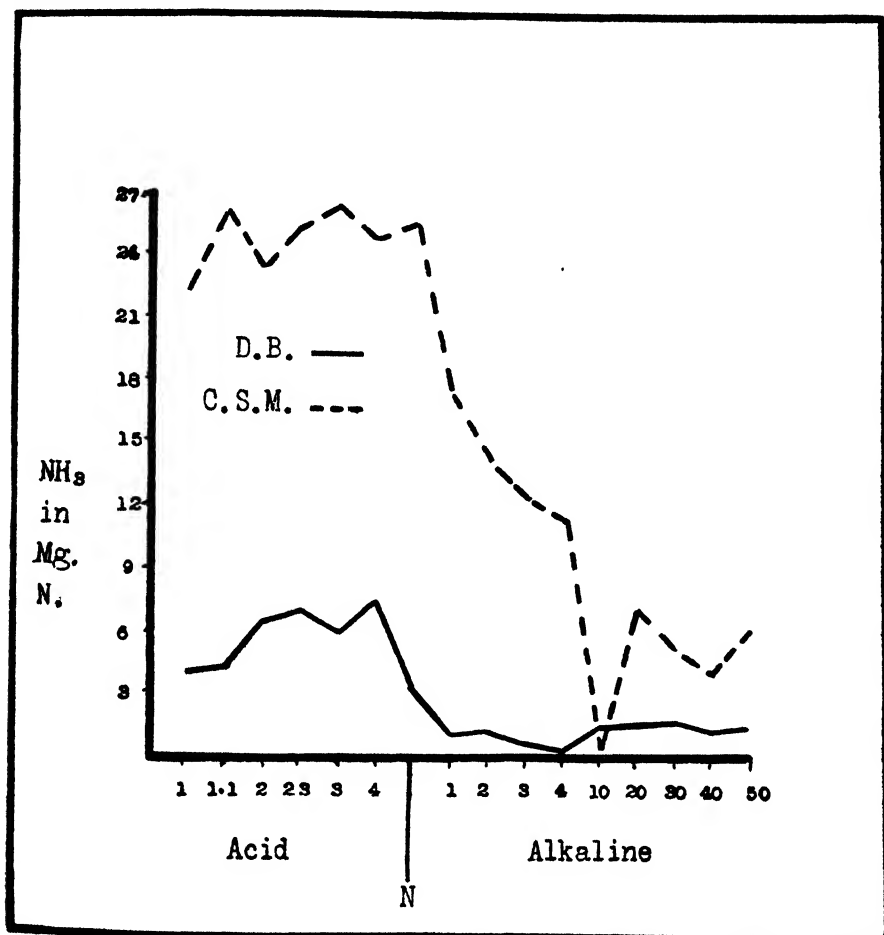


Fig. 9.—The effect of reaction on *Zygorrhynchus Vuilleminii* in Penn clay loam ( $H_2SO_4-CaCO_3$ ).

In Table XV and figure 10 are shown the effect of reaction on ammonification by *Rhizopus nigricans* (using 36,000 spores per 1 c.c.) in Norfolk sandy loam with dried blood as the source of organic matter. In increasing the acidity from the neutral point to 2,000 pounds there is a



TABLE XIV  
THE EFFECT OF REACTION ON ZYGORRHYNCHUS VUILLEMINII IN  
PENN CLAY LOAM  
(H<sub>2</sub>SO<sub>4</sub>—CaCO<sub>3</sub>)

No.	Organic Matter	Treatment	NH <sub>3</sub> accumulated in			Increase over check Mg. N.
			Mg. N.	Mg. N.	Av. Mg. N.	
	155 mg. N. Cottonseed Meal	Check .....	3.32	3.08	3.20	.....
217-218	"	Acid $\approx$ 1000 lbs. CaO....	26.01	25.40	25.71	22.51
219-220	"	Soil L. R. $\approx$ 1100 lbs. CaO	29.12	29.26	29.19	25.99
221-222	"	Acid $\approx$ 2000 lbs. CaO....	26.82	26.32	26.57	23.37
223-224	"	Acid $\approx$ 2300 lbs. CaO....	27.16	29.89	28.53	25.33
225-226	"	Acid $\approx$ 3000 lbs. CaO....	30.66	29.61	30.14	26.94
227-228	"	Acid $\approx$ 4000 lbs. CaO....	28.00	28.00	28.00	24.80
229-230	"	Neutral .....	28.14	29.47	28.81	25.61
231-232	"	Alk. $\approx$ 1000 lbs. CaO....	21.08	20.91	21.00	17.80
233-234	"	Alk. $\approx$ 2000 lbs. CaO....	17.25	17.53	17.39	14.19
235-236	"	Alk. $\approx$ 3000 lbs. CaO....	15.60	15.79	15.70	12.50
237-238	"	Alk. $\approx$ 4000 lbs. CaO....	14.87	14.60	14.74	11.54
239-240	"	Alk. $\approx$ 10,000 lbs. CaO...	3.06	3.99	3.53	0.33
241-242	"	Alk. $\approx$ 20,000 lbs. CaO...	10.43	10.08	10.26	7.06
243-244	"	Alk. $\approx$ 30,000 lbs. CaO...	8.05	9.10	8.53	5.33
245-246	"	Alk. $\approx$ 40,000 lbs. CaO...	7.21	7.49	7.35	4.15
247-248	"	Alk. $\approx$ 50,000 lbs. CaO...	9.38	Lost	9.38	6.18

TABLE XV  
THE EFFECT OF REACTION ON RHIZOPUS NIGRICANS IN NORFOLK SANDY LOAM  
(H<sub>2</sub>SO<sub>4</sub>—CaCO<sub>3</sub>)

No.	Organic Matter	Treatment	NH <sub>3</sub> accumulated in			Increase over check Mg. N.
			Mg. N.	Mg. N.	Av. Mg. N.	
	155 mg. N. Dried Bl'd	Check .....	2.53	2.27	2.40	.....
249-250	"	Acid $\approx$ 1000 lbs. CaO....	19.32	17.74	18.53	16.13
251-252	"	Acid $\approx$ 2000 lbs. CaO....	28.57	28.47	28.52	26.12
253-254	"	Soil L. R. $\approx$ 2300 lbs. CaO	13.16	14.49	13.83	11.43
255-256	"	Acid $\approx$ 3000 lbs. CaO....	10.22	12.88	11.55	9.15
257-258	"	Acid $\approx$ 4000 lbs. CaO....	8.75	9.52	9.14	6.74
259-260	"	Neutral .....	11.80	12.70	12.25	9.85
261-262	"	Alk. $\approx$ 1000 lbs. CaO....	8.40	9.10	8.75	6.35
263-264	"	Alk. $\approx$ 2000 lbs. CaO....	5.20	5.70	5.45	3.05
265-266	"	Alk. $\approx$ 3000 lbs. CaO....	7.30	5.25	6.78	4.38
267-268	"	Alk. $\approx$ 4000 lbs. CaO....	4.55	4.87	4.76	2.36
269-270	"	Alk. $\approx$ 10,000 lbs. CaO...	4.69	7.84	6.27	3.87
271-272	"	Alk. $\approx$ 20,000 lbs. CaO...	9.38	3.85	6.62	4.22
273-274	"	Alk. $\approx$ 30,000 lbs. CaO...	3.85	3.99	3.92	1.52
275-276	"	Alk. $\approx$ 40,000 lbs. CaO...	1.89	3.36	2.63	0.23
277-278	"	Alk. $\approx$ 50,000 lbs. CaO...	3.92	3.99	3.96	1.56

gradual and pronounced increase in ammonia. In the cottonseed meal series as shown in Table XVI and figure 10, the maximum ammonia accumulation (with but one exception) occurs between the neutral point and an acidity of 2,300 pounds. Above the latter point there is a decrease in ammonia with increasing acidity. From the neutral point to 4,000 pounds alkalinity there is a gradual decrease in ammonia (with one

exception) with a corresponding increase in alkalinity. From 10,000 to 50,000 pounds CaO there is too great a variation in the results to permit more than the assertion that there appears to be a tendency toward a decrease in ammonia with increasing alkalinity. McLean and Wilson (14) working with *Rhizopus nigricans* in a gravelly loam soil having a lime requirement of 1,200 pounds CaO per acre and using 155 mg. N in cottonseed meal found that the addition of 0.5 per cent and 2 per cent  $\text{CaCO}_3$  did not greatly affect ammonia production, though there was a slight tendency towards an increase. Naturally enough the organism employed by these investigators in all likelihood was of a different strain

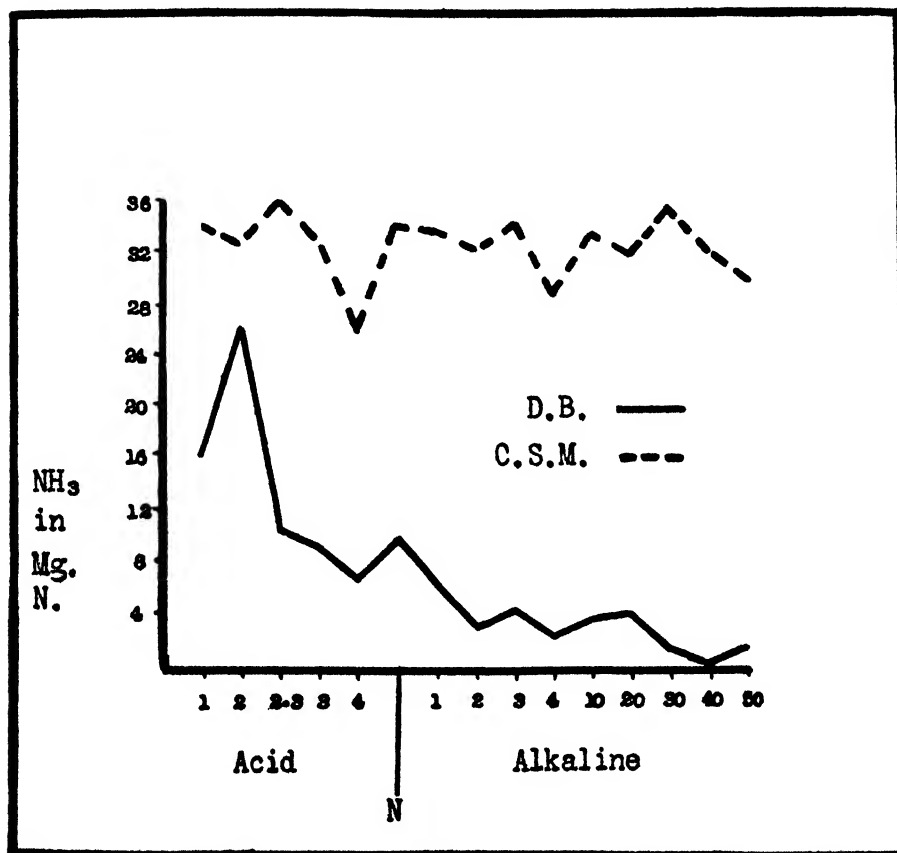


Fig. 10.—The effect of reaction on *Rhizopus nigricans* in Norfolk sandy loam ( $\text{H}_2\text{SO}_4$ — $\text{CaCO}_3$ ).

from that used in the present experiments and undoubtedly this would account for the divergence in results. The data obtained by McLean and Wilson (14) and those recorded in Table XVI are in agreement in showing that *Rhizopus nigricans* attains its maximum ammonia accumulation in a sandy soil having a neutral reaction. However, when the alkalinity

TABLE XVI  
THE EFFECT OF REACTION ON RHIZOPUS NIGRICANS IN NORFOLK SANDY LOAM  
( $H_2SO_4$ — $CaCO_3$ )

No.	Organic Matter	Treatment	NH <sub>3</sub> accumulated in			Increase over check Mg. N.
			Mg. N.	Mg. N.	Av. Mg. N.	
	155 mg. N. Cottonseed Meal	Check .....	2.91	2.69	2.80	.....
279-280	"	Acid $\approx$ 1000 lbs. $CaO$ ....	36.53	37.08	36.81	34.01
281-282	"	Acid $\approx$ 2000 lbs. $CaO$ ....	35.57	35.55	35.56	32.76
283-284	"	Soil L. R. $\approx$ 2300 lbs. $CaO$	39.27	38.22	38.75	35.95
285-286	"	Acid $\approx$ 3000 lbs. $CaO$ ....	37.24	35.14	36.19	33.39
287-288	"	Acid $\approx$ 4000 lbs. $CaO$ ....	28.56	29.12	28.84	26.04
289-290	"	Neutral .....	36.60	37.04	36.87	34.07
291-292	"	Alk. $\approx$ 1000 lbs. $CaO$ ....	35.88	36.83	36.36	33.56
293-294	"	Alk. $\approx$ 2000 lbs. $CaO$ ....	36.46	33.75	35.11	32.31
295-296	"	Alk. $\approx$ 3000 lbs. $CaO$ ....	36.82	39.34	37.08	34.28
297-298	"	Alk. $\approx$ 4000 lbs. $CaO$ ....	31.55	31.45	31.50	28.70
299-300	"	Alk. $\approx$ 10,000 lbs. $CaO$ ...	35.42	36.68	36.05	33.25
301-302	"	Alk. $\approx$ 20,000 lbs. $CaO$ ...	34.72	34.72	34.72	31.92
303-304	"	Alk. $\approx$ 30,000 lbs. $CaO$ ...	42.81	35.14	38.98	36.18
305-306	"	Alk. $\approx$ 40,000 lbs. $CaO$ ...	36.68	32.90	34.79	31.99
307-308	"	Alk. $\approx$ 50,000 lbs. $CaO$ ...	32.20	33.04	32.62	29.82

TABLE XVII  
THE EFFECT OF REACTION ON RHIZOPUS NIGRICANS IN PENN CLAY LOAM  
( $H_2SO_4$ — $CaCO_3$ )

No.	Organic Matter	Treatment	NH <sub>3</sub> accumulated in			Increase over check Mg. N.
			Mg. N.	Mg. N.	Av. Mg. N.	
	155 mg. N. Dried Bl'd	Check .....	2.73	2.87	2.80	.....
309-310	"	Acid $\approx$ 1000 lbs. $CaO$ ....	11.30	11.90	11.60	8.80
311-312	"	Soil L. R. $\approx$ 1100 lbs. $CaO$	11.06	11.20	11.13	8.33
313-314	"	Acid $\approx$ 2000 lbs. $CaO$ ....	10.22	10.57	10.39	7.59
315-316	"	Acid $\approx$ 2300 lbs. $CaO$ ....	10.78	9.80	10.29	7.49
317-318	"	Acid $\approx$ 3000 lbs. $CaO$ ....	11.20	11.20	11.20	8.40
319-320	"	Acid $\approx$ 4000 lbs. $CaO$ ....	9.66	9.17	9.41	6.61
321-322	"	Neutral .....	9.87	10.95	10.41	7.61
323-324	"	Alk. $\approx$ 1000 lbs. $CaO$ ....	8.90	9.95	9.43	6.63
325-326	"	Alk. $\approx$ 2000 lbs. $CaO$ ....	6.33	6.43	6.38	3.58
327-328	"	Alk. $\approx$ 3000 lbs. $CaO$ ....	4.81	4.97	4.89	2.09
329-330	"	Alk. $\approx$ 4000 lbs. $CaO$ ....	4.25	3.85	4.05	1.25
331-332	"	Alk. $\approx$ 10,000 lbs. $CaO$ ...	6.73	6.51	6.62	3.82
333-334	"	Alk. $\approx$ 20,000 lbs. $CaO$ ...	5.74	6.44	6.09	3.19
335-336	"	Alk. $\approx$ 30,000 lbs. $CaO$ ...	6.02	6.02	6.02	3.22
337-338	"	Alk. $\approx$ 40,000 lbs. $CaO$ ...	6.39	6.35	6.37	3.57
339-340	"	Alk. $\approx$ 50,000 lbs. $CaO$ ...	6.72	5.46	6.09	3.29

is increased beyond this point they find a tendency toward increased ammonia accumulation where the present work indicates that the general trend is towards a decrease in ammonia.

The effect of soil reaction on ammonification by *Rhizopus nigricans* in Penn clay loam, using dried blood is shown in Table XVII and figure 11. The maximum ammonification occurs between the neutral point and an acidity of 3,000 pounds, the differences between treatments being in-

significant. With an acidity of 4,000 pounds there is a decrease in ammonia. It is to be noted that in this experiment the number of spores used for inoculation was so small (32,000 per 1 c.c.) that the ammonia accumulation was comparatively slight, consequently the differences in treatment could hardly have been expected to be very striking. However, it is quite clearly demonstrated that with increasing alkalinity up to 4,000 pounds there is a corresponding decrease in ammonia. When the alkalinity is increased beyond this point, the phenomenon already referred to, namely a constant, makes its appearance.

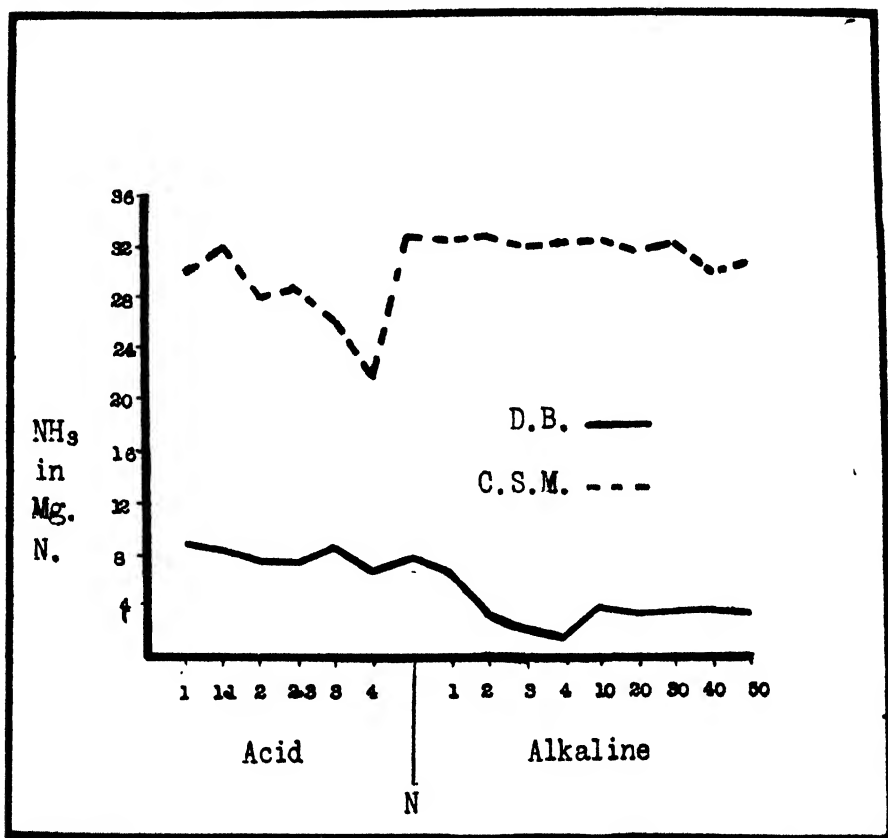


Fig. 11.—The effect of reaction on *Rhizopus nigricans* in Penn clay loam ( $\text{H}_2\text{SO}_4$ — $\text{CaCO}_3$ ).

In the cottonseed meal series as shown in Table XVIII and figure 11, the maximum ammonia accumulation occurs between the neutral point and an acidity of 1,100 pounds. With 2,000 and 2,300 pounds the amount of ammonia is somewhat lower, but with an increase in acidity beyond this point there is a corresponding decrease in ammonia. The addition of various amounts of  $\text{CaCO}_3$  in this instance resulted in practically no differences in ammonia accumulation. An explanation of this phenomenon

might depend upon the fact that in such a heavy soil,  $\text{CaCO}_3$  was not able to act sufficiently rapidly to overcome the acidity of the soil together with that produced by the by-products of the decomposition of cottonseed meal.

TABLE XVIII  
THE EFFECT OF REACTION ON RHIZOPUS NIGRICANS IN PENN CLAY LOAM  
( $\text{H}_2\text{SO}_4$ — $\text{CaCO}_3$ )

No.	Organic Matter	Treatment	NH <sub>3</sub> accumulated in			Increase over check Mg. N.
			Mg. N.	Mg. N.	Av. Mg. N.	
	155 mg. N. Cottonseed					
	Meal	Check .....	3.32	3.08	3.20	.....
341-342	"	Acid $\approx$ 1000 lbs. $\text{CaO}$ ....	33.69	33.33	33.51	30.31
343-344	"	Soil L. R. $\approx$ 1100 lbs. $\text{CaO}$	35.70	34.58	35.14	31.94
345-346	"	Acid $\approx$ 2000 lbs. $\text{CaO}$ ....	30.80	31.76	31.28	28.08
347-348	"	Acid $\approx$ 2300 lbs. $\text{CaO}$ ....	31.36	32.06	31.71	28.51
349-350	"	Acid $\approx$ 3000 lbs. $\text{CaO}$ ....	29.68	28.70	29.19	25.99
351-352	"	Acid $\approx$ 4000 lbs. $\text{CaO}$ ....	23.91	25.90	24.95	21.75
353-354	"	Neutral .....	37.10	36.96	37.03	33.83
355-356	"	Alk. $\approx$ 1000 lbs. $\text{CaO}$ ....	35.43	35.33	35.38	32.18
357-358	"	Alk. $\approx$ 2000 lbs. $\text{CaO}$ ...	35.54	36.18	35.86	32.66
359-360	"	Alk. $\approx$ 3000 lbs. $\text{CaO}$ ....	35.86	34.84	35.35	32.15
361-362	"	Alk. $\approx$ 4000 lbs. $\text{CaO}$ ....	35.18	35.58	35.38	32.18
363-364	"	Alk. $\approx$ 10,000 lbs. $\text{CaO}$ ..	36.54	35.14	35.84	32.64
365-366	"	Alk. $\approx$ 20,000 lbs. $\text{CaO}$ ..	34.86	Lost	34.86	31.66
367-368	"	Alk. $\approx$ 30,000 lbs. $\text{CaO}$ ..	36.68	33.88	35.28	32.08
369-370	"	Alk. $\approx$ 40,000 lbs. $\text{CaO}$ ..	33.46	32.76	33.11	29.91
371-372	"	Alk. $\approx$ 50,000 lbs. $\text{CaO}$ ..	34.58	33.39	33.99	30.79

Again, the facts cited above have the same general tendency as those previously referred to (14). For in the latter case using the same kind of soil as in the present instance (except for the fact that it was neutral), it was found that a high increase in alkalinity caused a depression in ammonia accumulation. Where dried blood was used instead of cottonseed meal, as a source of organic matter, the results were likewise in agreement. It is also of interest to note that the above investigators found that *Trichoderma Koningi* evidently requires a neutral medium for its best growth.

Considering the data which have been presented concerning the effect of reaction on ammonification by *Rhizopus nigricans* using both organic materials in sandy soil, the maximum ammonia accumulation takes place with a reaction between the neutral point and 2,300 pounds, while in Penn clay loam, due no doubt to its physical condition, an acidity of 3,000 pounds would represent the outer limit of maximum ammonia accumulation. In general, also, it may be stated (subject to the exceptions already noted) that an increase in alkalinity from 1,000 to 4,000 pounds  $\text{CaO}$  per acre causes a diminution in ammonia accumulated.

Considering in their entirety the data which have been presented concerning the effect of soil reaction on ammonification by these fungi where the reaction was altered by additions of  $\text{H}_2\text{SO}_4$  or  $\text{CaCO}_3$ , the following points are indicated.

1. Alteration of soil reaction has practically the same effect upon the three different fungi studied.
2. The effect of soil reaction is more pronounced where dried blood rather than cottonseed meal is employed as the source of organic nitrogenous matter to be ammonified.
3. The effect of reaction on ammonification by these fungi is more pronounced in clay than in sandy soil.
4. In general the maximum ammonia accumulation by these fungi in sandy or clay soils with either kind of organic matter, occurs between the neutral point and an acidity of 2,000 pounds.
5. An increase in application of  $\text{CaCO}_3$  causes a diminution in ammonia accumulation.

#### SUMMARY

Under the conditions of the experiment the following points have been established.

1. *Rhizopus nigricans*, *Zygorrhynchus Vuilleminii* and *Penicillium* sp. 10 are all influenced in the same way by any specific changes in soil reaction. They possess a comparatively narrow range of reaction tolerance for maximum ammonification which was found to be between the neutral point and an acidity equivalent to 2,000 pounds  $\text{CaO}$  per acre. In general, an acidity greater than 2,000 pounds caused a depression in ammonification as did an increase in alkalinity beyond the neutral point.
2. It is significant that the results obtained were practically the same whether sandy or clay soils (having either high or low lime requirements) were used with either dried blood or cottonseed meal.
3. Where normal solutions of  $\text{HCl}$  and  $\text{NaOH}$  were used to alter soil reaction, the data were somewhat more concordant than where  $\text{H}_2\text{SO}_4$  and  $\text{CaCO}_3$  were used for the same purpose.
4. There is good reason then to believe that the practical significance of this experimentation points to the fact that where the soil reaction is unfavorable for the activities of the soil bacteria concerned in ammonification, the soil fungi might prove to be an important compensating factor in maintaining fertility.

In conclusion it is a privilege to express appreciation to Dr. J. G. Lipman for his helpful suggestions ever at the writer's disposal, and to Dr. M. T. Cook and Professor J. P. Helyar for their kind assistance.

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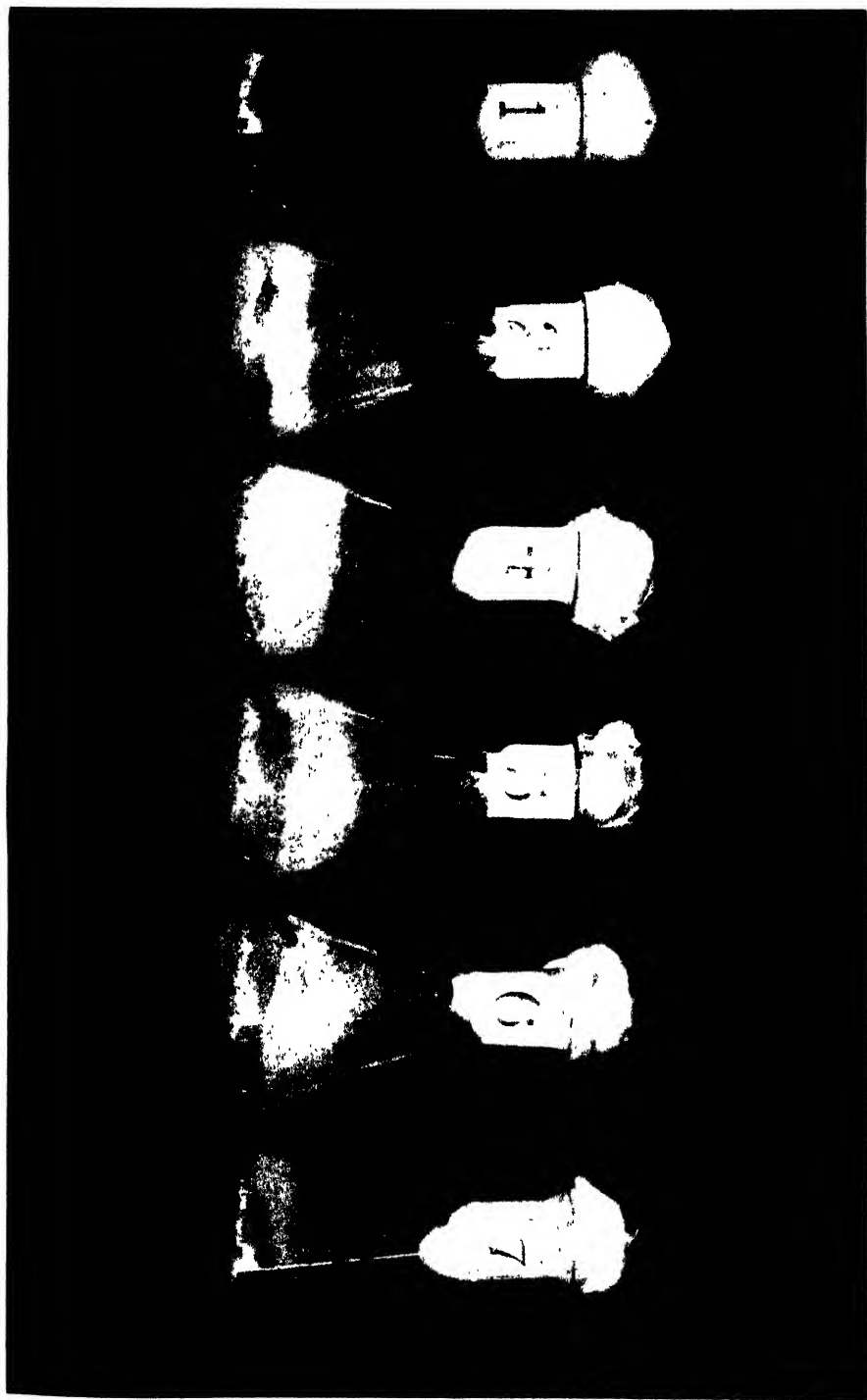


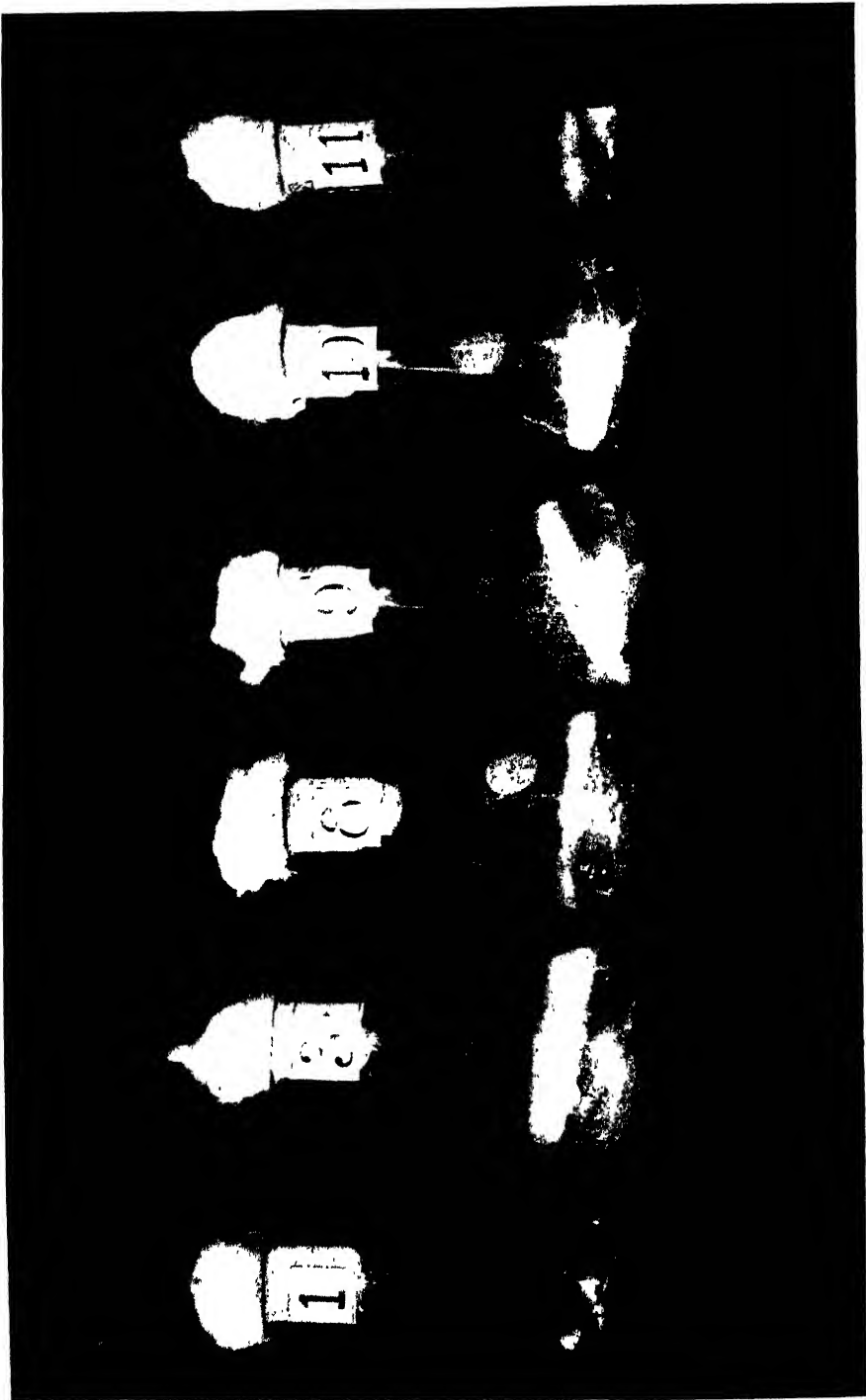
## PLATE I

The effect of soil reaction on mycelial growth of *Rhizopus nigricans* using dried blood as the source of organic matter.

HCl(N/1) added in amounts equivalent to:

1. Check.
2. Original soil acid  $\approx$  400 lbs. CaO per acre.
4. Acid  $\approx$  1000 lbs.
5. Acid  $\approx$  2000 lbs.
6. Acid  $\approx$  3000 lbs.
7. Acid  $\approx$  4000 lbs.





## PLATE II

The effect of soil reaction on mycelial growth of *Rhizopus nigricans* using dried blood as the source of organic matter.

NaOH(N/1) added in amounts equivalent to:

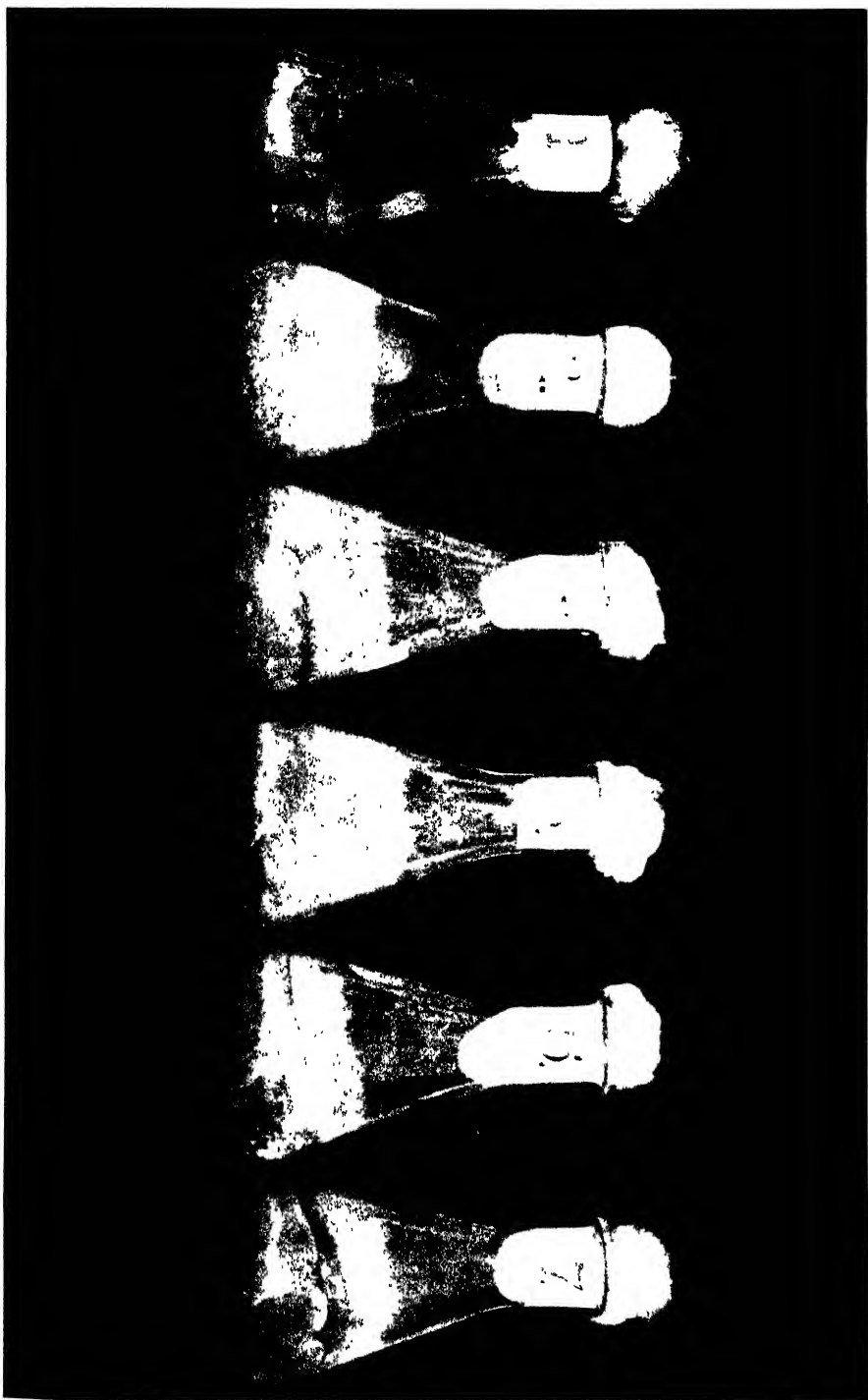
1. Check.
2. Neutral.
8. Alk.  $\approx$  1000 lbs. CaO per acre.
9. Alk.  $\approx$  2000 lbs.
10. Alk.  $\approx$  3000 lbs.
11. Alk.  $\approx$  4000 lbs.

### PLATE III

The effect of soil reaction on mycelial growth of *Rhizopus nigricans* using cotton-seed meal as the source of organic matter.

HCl(N/1) added in amounts equivalent to:

1. Check.
2. Original soil acid  $\approx$  400 lbs. CaO per acre.
4. Acid  $\approx$  1000 lbs.
5. Acid  $\approx$  2000 lbs.
6. Acid  $\approx$  3000 lbs.
7. Acid  $\approx$  4000 lbs.





#### PLATE IV

The effect of soil reaction on mycelial growth of *Rhizopus nigricans* using cotton-seed meal as the source of organic matter.

NaOH(N/1) added in amounts equivalent to:

1. Check.
3. Neutral.
8. Alk.  $\approx$  1000 lbs. CaO per acre.
9. Alk.  $\approx$  2000 lbs.
10. Alk.  $\approx$  3000 lbs.
11. Alk.  $\approx$  4000 lbs.





# ACCUMULATION OF SALTS IN OHIO SOILS<sup>1</sup>

By

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This phenomenon is not of common occurrence in soils of the humid regions where the rainfall is sufficiently distributed throughout the year to enable the gravitational water to sweep downward any excessive amount of soluble minerals which have been carried toward the surface by capillary water. While the accumulation of soluble salts at or near the surface forming alkali deposits is common in semi-arid and arid sections, no similar occurrences in humid soils have been reported so far as we know, except by Cameron.<sup>2</sup> He reports alkali spots observed by Dr. Whitney at the Maryland Experiment Station and near Starke, Bradford County, Florida. The formation in Maryland contains about 2 per cent of water-soluble salts. About 50 per cent of the material was calcium nitrate and 90 per cent was in the form of nitrates.

The crust found in Florida was composed chiefly of sodium chloride; sulphates and phosphates were also present in measurable quantities. Accumulations consisting chiefly of sodium chloride were reported in Mississippi, Louisiana and Texas. Deposits of soluble sulphates which were considered to be due to the oxidation of iron sulphide are also reported as having been found in Maryland and New York.

## CASES OBSERVED IN OHIO

So far as they have been observed, the areas in Ohio affected in this way are located in the southern part of Highland County and in Brown and Clermont Counties where the loess soils overlie the Illinois glaciation. The underlying rock in this section is limestone which is covered by from 10 to 25 feet of boulder clay. Leverett<sup>3</sup> reports that occasional exposures of residual clays between the blue till and the rock are found.

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<sup>1</sup> Received for publication May 11, 1916.

<sup>2</sup> Cameron, F. C. Soil solutions. U. S. Dept. Agr. Bur. Soils, Bul. 17, 39 p., 1901.

<sup>3</sup> Leverett, F. Glacial formations and drainage features of the Erie and Ohio Basins. U. S. Geol. Survey Monograph 41, 1902.

Orton<sup>4</sup> states that occasionally black, mucky clay lies immediately below the blue till and a few feet above the rock. In this same report reference is made to a deposit of soil and bog iron ore between the yellow and blue till which is said to extend over an area of several miles. The information available concerning the geology of this section does not furnish any explanation as to the source of the excess of salts.

Where this condition was found the deposition of soluble salts carried to the surface by capillary waters forms a noticeable efflorescence resembling frost on the soil. The excessive salt concentration of the soil water is also indicated by a white coating on the sparse vegetation, mostly weeds, growing on these spots.

The soils on which the deposits were observed are very poorly drained. Indications of the excess of water were seen in the numerous workings of crayfish found in the locality. It is stated by the owners of land where these formations occur that they are most pronounced after a heavy rainfall. This indicates that the subsoil water is strongly impregnated with salts, for when a connection is established between the saline subsoil water and the water evaporating from the surface, a capillary rise of salts takes place followed by a crystallization at the surface.

#### THE SALT CONTENT OF WELL-WATER

The fact that the water of shallow wells which are from 8 to 12 feet deep is strongly impregnated with salts of calcium and magnesium also furnishes further evidence that the subsoil water holds excessive amounts of these salts in solution. The water from a shallow well about one mile from one of the soils examined contained 3.59 gm. total solids per liter.<sup>1</sup> The amounts of calcium, magnesium and sulphate found were equivalent to 1.65 gm. of calcium sulphate ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ) and 3.76 gm. magnesium sulphate ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ). No aluminum or iron was found in the water. Calcium sulphide is reported in artesian water at Ripley, Brown County, as 14.9 grains. This water also contains calcium hyposulphite 2.58 grains per gallon.<sup>2</sup>

#### COMPOSITION OF SALT FORMATION

Water extracts of one of the soils on which deposits of salts were observed and of adjacent soil which appeared to be free from salts were obtained by extracting 50 gm. of the surface soil with 1000 c.c. of water. The results expressed as per cent in soil show that magnesium sulphate and aluminum sulphate were the chief constituents; only a slight trace of calcium was found.

<sup>1</sup> Orton, E. Geology of Clermont County. In Rpt. Geol. Survey Ohio, v. 1 p. 443, 1875.

<sup>2</sup> Leverett, F. Water resources of Indiana and Ohio. In U. S. Geol. Survey, 18th Ann. Rpt., pt. 4, p. 496, 1897.

The magnesium, aluminum and sulphate found are equivalent to 4.27 per cent  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  and 4.90 per cent  $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$ . These figures indicate an excessive accumulation of salts in soils receiving about 30 inches of rainfall annually.

Although the water extract of adjacent soil, the surface of which appeared to be free from deposit of salts, contained a much smaller amount of soluble salts, the quantities of aluminum and sulphate found indicate the presence of an appreciable accumulation of aluminum sulphate. The reaction of the water extract of the soil was decidedly acid, the acidity being due to the considerable quantity of aluminum sulphate present.

In another locality where a similar accumulation of salts was found at the surface, the soil was sampled in one-foot sections to a total depth of 6 feet. A qualitative examination of the samples representing the several depths showed the presence of large amounts of water-soluble sulphate and calcium, except in the sample taken to a depth of 3 feet, which gave no test for calcium in the water solution.

TABLE I  
COMPOSITION OF WATER EXTRACTS OF SOILS

	Soil including salt deposit at surface	Adjacent soil
MgO .....	.695	.022
$\text{Al}_2\text{O}_3$ .....	.753	.178
$\text{SO}_3$ .....	3.845	.084
Cl .....	.120	trace

The sample from the surface contained considerable aluminum sulphate, but none of the lower depths showed a trace of aluminum. The water extract of the surface soil in this case was also strongly acid and that of the other depths was neutral in reaction. No water-soluble magnesium was present in this soil as compared with the first soil described, the soluble salt content of which was composed chiefly of magnesium and aluminum sulphates and contained only a small amount of calcium. No water-soluble iron was found in either case. Many iron concretions were found in soils in this vicinity.

The salts which form the main mass of the saline deposits forming the so-called alkali soils of arid regions generally include carbonates, chlorides and sulphates of sodium, potassium, calcium and magnesium. The composition of the residue on the soils described differs from these alkali soils in that the salts are either the sulphate of calcium or magnesium, together with considerable amounts of aluminum sulphate, which imparts a very strong acid reaction to the surface soil extract, as indicated by phenolphthalein and litmus paper.

The presence of aluminum sulphate can be explained as being due to the absorption of calcium or magnesium from their salts, leaving the sul-

phate radical free to combine with aluminum. Oxidation of pyrites in the soil or subsoil may be a contributing cause of the accumulation of sulphates of calcium and magnesium observed in these cases.

The lack of vegetation on the areas affected may not be due altogether to the presence of salts, although this is indicated, but to poor drainage. The soil being very impervious, it is a question whether the remedies which suggest themselves, namely: drainage and liming, will overcome the difficulty. More information as to the geological formation and a more extended study of the subsoil is necessary before the probable source of the salts can be determined.

# THE YIELD AND NITROGEN CONTENT OF SOYBEANS AS AFFECTED BY INOCULATION<sup>1</sup>

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Soybeans lend themselves readily to comparisons of inoculating material derived from different sources. It appears that this crop is less likely to become inoculated spontaneously than other legumes which may be used in tests of the value of commercial cultures for soil inoculation. Moreover, soybeans are a satisfactory crop for the purpose just indicated, since the plants are rather hardy and may be made to grow without difficulty under a wide range of soil and climatic conditions.

The data recorded in the following pages relate to a comparison of commercial cultures as well as of soil derived from different sources. The experiments were carried out by means of 1-gallon, glazed earthenware pots. The soil employed was so treated as to make the nitrogen supply the limiting factor of growth. Two series, each containing 26 pots, were included in the experiment.

In the first of these, to be designated as Series A, there was employed a rather poor sandy loam soil possessing a distinct acid reaction. The entire amount of soil used in this series was thoroughly mixed and distributed in quantities of 10½ pounds each in the glazed earthenware pots. There were then added to the soil in each pot, and thoroughly mixed with it, 2 gm. of acid phosphate, 1 gm. of muriate of potash and 10 gm. of ground limestone. Optimum moisture conditions were established by the addition of water, and 15 seeds of the Guelph variety of soybeans were planted in each pot on June 24, 1915. Inoculation was then provided, or left out, according to the following scheme:

POTS	INOCULATION
1- 2	Check.
3- 4	Nitragin.
5- 6	Farmogerm.
7- 8	Mulford Nitrogerm.
9-10	Standard Nitrogerm.
11-12	Ferguson's Composite.
13-14	Bacto-Natural.
15-16	Soybean Soil, New Jersey Agricultural Experiment Station.
17-18	Soybean Soil, Middlesex County, New Jersey.
19-20	Cowpea Soil, Mercer County, New Jersey.
21-22	Soybean Soil, Atlantic County, New Jersey.
23-24	Soybean Soil, Sussex County, New Jersey.
25-26	Sporogen (old sample).

<sup>1</sup> Received for publication April 10, 1916.

Of the inoculating material named above, Nitragin was furnished by the German-American Nitragin Company, of Milwaukee, Wis.; Farmogerm, by Earp-Thomas Farmogerm Company, Bloomfield, N. J.; The Mulford Nitrogerm, by the H. K. Mulford Company, Glenolden, Pa.; the Standard Nitrogerm, by the Standard Nitrogerm Company, Glen Ridge, N. J.; Ferguson's Composite, by the Homewood Nitrogen Company, New York City; the Bacto-Natural, by Lewis Sturtevant Woodruff, of Lexington, Mass.; Sporogen, by Bruno Grosche & Co., of New York City. The last named was an old sample which had been kept in the laboratory for several years. The results secured from it should not for this reason, be accepted as a correct indication of the value of this material for inoculating purposes. It was included in the test for the purpose of determining whether positive results may be obtained from it even though it had been kept in a dry condition in the laboratory for several years.

Where inoculation was made by means of soil from different sources, an infusion was prepared in each case and equivalent quantities of such infusion were used for inoculation. The soybean plots of the Experiment Station from which a part of the inoculation material was derived have grown soybeans for several years and seem to be well supplied with bacteria capable of producing nodules on these plants. The Middlesex County soil had grown soybeans, some of which at least were known to have been inoculated. The soil from Mercer County had grown a good crop of cowpeas whose roots were well supplied with nodules. The soil from Atlantic County was claimed to have grown soybeans. There was no definite record, however, as to the facts in the case, particularly as to whether plants actually grown on the land in question had been inoculated. The soil from Sussex County had grown a good crop of soybeans whose roots were well supplied with nodules.

The seed germinated well and a good stand of plants was secured in each case. The crop was harvested on September 18, 1915, dried and weighed, and the weights recorded. The dried samples were ground and portions of the ground material were used for nitrogen determinations by the Kjeldahl method. The results secured are recorded in Table I.

On examining the data in question, we find that there is, with few exceptions, a very satisfactory agreement in the duplicates of each treatment. The check pots produced, on an average, 8.25 gm. of dry matter, whereas the inoculated soils produced, in several instances, more than twice as much dry matter. It will be observed that Nitragin, Farmogerm and the soil infusion from the Sussex County soil were particularly effective in providing for large yields. Bacto-Natural, the soil infusion from the Mercer County soil, the soil infusion from the Atlantic County soil and Sporogen did not, apparently, inoculate the soil sufficiently to

provide for an increased growth. The yield of dry matter for the soils inoculated with Ferguson's Composite was, on the average, but little greater than that from the checks.

TABLE I

RECORD OF DRY MATTER AND NITROGEN OBTAINED FROM SOYBEANS GROWN IN INOCULATION TESTS: SERIES A

No.	Inoculation	Dry Matter gm.		Inc. over check gm.	Nitrogen %	Tot. Nitrog'n mg.		Inc. over ch'k mg.
		p'rPot	Aver.			p'rPot	Aver.	
1	.....	10.5			1.299	136		
2	Check .....	6.0	8.25		1.358	82	109	
3	.....	22.0			3.282	722		
4	Nitragin .....	20.5	21.25	13.00	3.312	679	700	591
5	.....	17.5			3.371	589		
6	Farmogerm .....	21.0	19.25	11.00	3.331	700	645	536
7	.....	15.0			3.272	491		
8	Mulford Nitrogerm .....	13.0	14.00	5.75	3.412	444	468	359
9	.....	18.0			3.411	614		
10	Standard Nitrogerm .....	13.8	15.90	7.65	3.480	480	547	438
11	.....	7.0			2.906	203		
12	Ferguson's Composite .....	12.0	9.50	1.25	2.836	340	272	163
13	.....	7.2			1.884	136		
14	Bacto-Natural .....	6.0	6.60	.....	1.765	106	121	12
15	.....	15.0			3.212	482		
16	Soybean Soil, N. J. Agr. Exp. Sta. ....	22.0	18.50	10.25	3.074	676	579	470
17	.....	16.0			3.106	497		
18	Soybean Soil, Mid. Co., N. J. ....	14.0	15.00	6.75	2.717	380	439	330
19	.....	7.4			1.329	98		
20	Cowpea Soil, Mer. Co., N. J. ....	10.0	8.70	0.45	1.933	193	146	37
21	.....	4.5			1.805	81		
22	Soybean Soil, Atl. Co., N. J. ....	7.3	5.90	.....	1.735	127	104	...
23	.....	18.0			3.312	596		
24	Soybean Soil, Sus. Co., N. J. ....	26.0	22.00	13.75	3.341	869	733	624
25	.....	8.0			1.458	117		
26	Sporogen (Old Sample).....	12.0	10.00	1.75	2.627	315	216	107

The yields of dry matter gain in interest when taken in conjunction with the percentages of nitrogen in the dry matter. It will be noted that in the checks, as well as in the soils treated with Bacto-Natural and the infusions from the Mercer County and Atlantic County soils, the percentage of nitrogen in the dry matter was below 2 per cent. On the other hand, in the dry matter of the plants which had been inoculated with Nitragin, Farmogerm, Mulford Nitrogerm, Standard Nitrogerm and the infusion from the Experiment Station plots and the Sussex County soil, the percentage of nitrogen was well above 3 per cent. It is clear, therefore, that soybean plants, devoid of inoculation, not only fail to produce a large yield of dry matter when the soil is deficient in available nitrogen, but also contain a much smaller proportion of nitrogen in the plant substance than is usually found in plants that are properly inoculated. It may be pointed out, also, that there were marked differences in the effectiveness of the different cultures as well as of the different soils employed as inoculating material. Among the commercial cultures used, Nitragin



TABLE II  
RECORD OF DRY MATTER AND NITROGEN OBTAINED FROM SOYBEANS GROWN  
IN INOCULATION TESTS: SERIES B

No.	Inoculation	Dry Matter gm.		Inc. over check gm.	Nitro- gen %	Tot. Nitrog'n mg.		Inc. over ch'k mg.
		p'rPot	Aver.			p'rPot	Aver.	
1	.....	30.0			3.296	989		
2	Check .....	27.0	28.50	.....	3.374	911	950	...
3	.....	29.0			3.572	1037		
4	Nitragia .....	23.0	26.00	.....	3.582	824	931	...
5	.....	27.6			3.611	996		
6	Farmogerm .....	26.8	27.20	.....	3.928	1053	1025	75
7	.....	34.0			3.434	1168		
8	Mulford Nitrogerm .....	24.0	29.00	0.50	3.621	869	1019	69
9	.....	30.0			3.582	1075		
10	Standard Nitrogerm .....	28.0	29.00	0.50	3.327	931	1003	53
11	.....	31.0			3.327	1031		
12	Ferguson's Composite .....	31.5	31.25	2.75	3.582	1129	1080	130
13	.....	25.5			3.552	906		
14	Bacto-Natural .....	28.0	26.75	.....	3.395	950	928	...
15	.....	28.0			3.505	981		
16	Soybean Soil, N. J. Agr. Exp. Sta. ....	28.3	28.15	.....	3.464	980	981	31
17	.....	29.0			3.385	982		
18	Soybean Soil, Mid. Co., N. J. ....	29.0	29.00	0.50	3.464	1005	994	44
19	.....	28.0			3.483	975		
20	Cowpea Soil, Mer. Co., N. J. ....	28.0	28.00	.....	3.532	989	982	32
21	.....	28.0			3.405	953		
22	Soybean Soil, Atl. Co., N. J. ....	24.3	26.15	.....	3.405	827	890	...
23	.....	32.5			3.453	1123		
24	Soybean Soil, Sus. Co., N. J. ....	26.0	29.25	0.75	3.462	900	1012	62
25	.....	31.0			3.505	1086		
26	Sporogen (Old Sample) .....	37.0	34.00	5.50	3.327	1230	1158	208

was evidently the most effective inoculating material; while, among the soil infusions employed, that derived from the Sussex County soil was the most effective. It may be safe to state, therefore, that commercial cultures may be fully as effective for inoculating purposes as suitable soil material, but that, under favorable conditions, soil material may prove to be fully as satisfactory as the best artificial cultures.

Another series, designated as Series B, was arranged to correspond to series A, except that the pots were filled with a silt loam soil in a good state of fertility and well provided with organisms capable of producing nodules on the roots of soybeans. The soil employed in this series had been utilized for the growing of soybeans in connection with certain plant-breeding experiments conducted by the Botanist of the Experiment Station. In this case 9 pounds of soil were placed in each pot and the optimum moisture conditions were established by the addition of water. Fifteen seeds of the Guelph variety of soybeans were planted in each pot on June 24, 1915. As in Series A, 2 gm. of acid phosphate, 1 gm. of muriate of potash and 10 gm. of ground limestone were added to and thoroughly mixed with the soil in each pot previous to the planting of the soybean seed. The crop was harvested on September 18, 1915.

The weight of the dry matter and the percentages of nitrogen in the dry matter, as found in each case, are recorded in Table II.

The yields, as recorded in this table, show that the soil employed was well supplied with the proper strain of *Bacillus radicicola*. The average yield of dry matter in the check pots was 28.50 gm. as against 8.25 gm. where soil lacking in these bacteria was employed. It appears, then, that the use of soil already inoculated would prevent the production of larger yields of dry matter where commercial cultures or soil infusions were employed. Theoretically, a further increase would have been possible only if the organisms introduced by the commercial cultures or the soil infusions were more efficient as nitrogen-fixers than those already present in the soil. A careful study of the data presented in Table II shows that the use of artificial culture material or of soil infusions did not lead to any striking increase in the yields of dry matter. Indeed, it may almost be assumed that the differences noted were within the limit of experimental error.

The same relations appear seemingly in the proportions of nitrogen present in the dry matter from the different pots. In all cases, the content of nitrogen in the dry matter was well above 3 per cent, and in several instances it was above  $3\frac{1}{2}$  per cent. It appears, further, that a slight, but none the less distinct, increase in the yield of total nitrogen was obtained from pots where Farmogerm, Mulford Nitrogerm, Standard Nitrogerm and Ferguson's Composite were employed. The pots in which some of the soil infusions were used also gave slight increases. The largest increase for the inoculated pots was obtained from soils which had been inoculated with an old sample of Sporogen. The average yield of dry matter from pots 25 and 26, where Sporogen was used, was 34 gm. However, this relatively high average was due largely to the yield of 37 gm. of dry matter obtained from Pot No. 26. Considering the data as they stand, there is hardly any justification for assuming that the organisms introduced by the Sporogen were responsible for the increase observed.

#### SUMMARY

Taking the data in their entirety, we are led to conclude that the use of inoculating material may be very desirable in the growing of soybeans, and perhaps of other legumes. The results recorded here confirm results previously recorded by our own station or by other stations. It appears that where the soil is lacking in the right type of *Bacillus radicicola*, inoculation is eminently desirable, and that, even where the organisms are present in limited numbers, the addition of larger numbers may be profitable. It appears, further, that there is a marked difference in quality of different commercial preparations for soil inoculation and that soils de-

rived from different sources may vary as widely as, though not more widely than, commercial cultures as to their effectiveness in promoting nitrogen fixation by legumes.

The authors take this opportunity of expressing appreciation for assistance rendered by Mr. H. C. McLean and Mr. L. K. Wilkins of the Department of Soils of this station.

## STUDIES ON SOIL COLLOIDS

### I. FLOCCULATION OF SOIL COLLOIDAL SOLUTIONS<sup>1</sup>

By M. I. WOLKOFF, *Michigan Agricultural College*

#### INTRODUCTION

The present status of our knowledge of flocculation of soil particles is based largely upon three sources of information, namely: (a) deductions from general colloidal chemistry, (b) studies with kaolin, and (c) studies with different clays.

From theoretical considerations the deductions from facts established with pure colloidal solutions are very valuable and should serve as guides in further investigations with such a complex medium, the soil.

Selmi (38) and Graham (16) have observed that salts and acids added to colloidal solutions cause its coagulation. Further, it was noticed that only electrolytes bring about coagulation, while non-electrolytes do not (5) possess this property at all, or only to a very small degree (30) when present in concentrated solutions. Later it was observed that some colloids, if placed in an electric field, move toward the cathode, while others gather themselves around the anode. According to this action they are classified as positive and negative colloids, respectively. Hardy (18) established the fact that the iron which coagulates a given colloid moves toward the opposite pole from the one to which the colloid moves. Linder and Picton (25) found that coagulation of colloids with negative electric charges is accompanied by the absorption of the positively charged ions of the electrolyte.

Besides the electrolytes and the familiar action of heat and frost (33), as discussed by Ostwald, there are other agencies which influence the stability of colloidal solutions. Several cases on record (40, 41, 14) showing that, when two different colloidal solutions with opposite electric charges are mixed together, the coagulation takes place. Radium rays help considerably in the coagulation of colloidal  $\text{Fe}(\text{OH})_3$  (23) by minute quantities of electrolytes which, if acting alone, are too dilute to cause a coagulation. Later it was found (31) that without the aid of an electrolyte, light from different sources acts as a slow coagulant, resembling in its behavior a weak electrolyte. Von Veimann and Alekseyev (42) have demonstrated that several, both positively and negatively charged, colloids can be coagulated at will by merely shaking a given colloidal solution for a sufficient length of time with the insoluble liquids or solids.

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<sup>1</sup> Received for publication May 3, 1916.

<sup>2</sup> The experimental results are taken from the author's thesis presented to the faculty of Michigan Agricultural College as a partial fulfillment of the requirement for the degree of M.Sc.

The foregoing are a few of the facts from colloidal chemistry which are helpful guides in understanding the phenomenon of flocculation in the soil colloidal solutions. The direct investigations in such solutions, however, are of more value for the investigator of soils because of the fact that the properties of even pure colloidal solutions vary greatly. Certainly, the variations increase enormously when one deals not with a solution of a single colloid but with a mixture of several colloids, in addition containing numerous salts in the true solution. In such a case the resultant of all these factors must be taken into consideration.

In order to throw light upon the properties of soil colloids a number of workers studied suspensions of kaolin, while others studied different clay suspensions either alone or in parallel with kaolin and other suspensions.

As early as 1866, or only a few years after the publication of Graham's classical investigations on colloidal substances, Schulze (36) recorded some of his results on the calcium and magnesium salt requirements for flocculation of clay suspensions. Later Schloessing (35) worked along the same line. Durham (12, 13) made an interesting discovery that although it requires a very small amount of sulphuric acid to flocculate the suspension of white clay (kaolin?), on further additions of sulphuric acid he reached the point when suspension did not clarify for a long time. Now, if to this mixture of clay suspension and sulphuric acid he added either more acid or some water, the suspension clarified quickly. Evidently, there is an equilibrium between the ions of true solution and the solid particles of clay. The flocculating action of sodium carbonate, on the other hand, continued to increase with the increase in concentration.

While working on the method of mechanical analysis, Hilgard (20, 21) noticed that clay suspension coagulated on passing through the narrow glass tube and flocculation is approximately inversely proportional to the size of the particles. A moderate increase in temperature decreased the flocculation in his case. He also studied the effect of lime on the texture of clays (19). Brewer (10) found the different clay suspension to be of different stability. In fact, some suspensions settle within a few days, while others remain turbid at the end of seven years, when kept at nearly the same temperature and in a quiet place. The acids he found to flocculate more quickly than the salts. Barus (5) in 1888 observed that non-electrolytes retard the clearing of suspensions. Later (6) he tested the hypothesis that the hydration of clay or kaolin particles is responsible for keeping their particles in suspension and came to the conclusion that such is not the case. He determined the densities of tripoli and bole in both water and ether and found them to be the same in both liquids. Since tripoli has practically the same density as quartz,

and bole approaches that of kaolin, he justified his conclusion on these grounds. Spring (39) noticed that the clearing power of salt depends upon the valence of the salt and the cation of the electrolyte, confirming in part the quantitative formula of Schulze (37) that the coagulating power of trivalent cation: divalent: monovalent as 350:20:1.

Bodländer (9) also measured the power of different salts for clearing the kaolin suspensions. Quincke (34) from his studies on pure colloids and kaolin suspensions advanced a theory on coagulation which in short implies the change in surface tension between the liquid and the oily substances. He claims to have observed oily films around the solid particles. Hall and Morison (17) while studying the efficiency of electrolytes in flocculating the kaolin suspensions found that the order of efficiency of acids to be  $\text{HCl} > \text{HNO}_3 > \text{H}_2\text{SO}_4$ . In the case of cations of salts it is  $\text{Al} > \text{Ca} > \text{K} > \text{Na}$ . Acids are better coagulants than their salts. Exceptions are  $\text{Al}_2(\text{SO}_4)_3$ , which is equal to  $\text{H}_2\text{SO}_4$ , but does not exceed it. Maschhaupt (29) found that  $\text{NaOH}$  stabilizes soil suspensions at low concentrations, while, if present above .015 N, it causes flocculation. Similar results were obtained with  $\text{Na}_2\text{CO}_3$  in which case the coagulation begins above 0.16 N. Oden (32) in rather extensive studies with peat colloidal solutions used  $\text{NaCl}$  for the flocculation. He had to saturate his colloidal solution with the pure salt and allow it to stand for 24 hours in order to bring about flocculation. McGeorge (28) working with suspensions of Hawaiian clays obtained results similar to those of Hall and Morison with the exceptions that he found  $\text{Al}_2(\text{SO}_4)_3$  to be the best flocculant among both salts and acids and the order of efficiency of strong acids was  $\text{HNO}_3 > \text{HCl} > \text{H}_2\text{SO}_4$ .

This short review of the past investigations on coagulation or flocculation, the term generally used in soil investigations, does not claim completeness. It reveals, however, a striking fact that practically all the work along this line has been done either with clays or kaolin. The later substance, which is very unplastic, crystalline in nature and remains in suspension only for a short time, can hardly be classified with the colloidal substances. No record of any importance was found in the literature bearing on the attempts to study suspensions of other soils besides clays. Yet it is a well known fact that no two colloidal solutions possess exactly the same properties toward the action of an electrolyte, and this is much more striking in the case of soil colloidal solutions, for undoubtedly one deals not with a single colloidal solution but with a mixture of several of them. The relation of one colloidal substance to another in such a mixture must be different with different soils, depending on the origin of the soil, its chemical composition, age, climate, etc. For this reason it was considered of sufficient importance to study the behavior of different classes of soils with respect to different electrolytes in order to better understand the phenomenon of flocculation in the soil.

## EXPERIMENTAL

*Method.* The soil colloidal solutions were prepared by adding to a bulk of fresh soil about 10 times its weight of distilled water, shaken well and allowed to stand over night. Then, the supernatant liquid was siphoned off and centrifuged at the rate of 2000 revolutions a minute for 15 minutes. The resultant solution would stand for several weeks and even months without appreciable sedimentations. In most of the experiments here recorded the same solutions were used. The exceptions will be mentioned later.

NATURE OF SUSPENSIONS USED

N. Soil Used	Reaction of soil with litmus paper	Dry Matter per 100 c.c. of suspension	Freezing point depression of solution
1. Brickyard clay (subsoil) ..	neutral	.3633	.003
2. Miami silt loam .....	neutral	.0700	.002
3. Clyde silt loam .....	neutral	.0913	.003
4. Muck .....	neutral	.0274	.002
5. Brickyard clay (soil) ....	neutral	.8098	....
6. Peaty muck .....	neutral	.0338	....
7. Kaolin .....	.....	.0247	....

The bacterial action in the colloidal solutions during the experiment was not controlled.

The acid, salt and alkali solutions were N/5 in strength and were the same throughout the experiments.

*Experiment I.* Qualitative test of electrolytes for flocculation of colloidal solutions.

In this experiment to 5 c.c. of suspension was added 5 c.c. of N/5 electrolyte, shaken vigorously for a short time and allowed to stand over night. Five positive signs \*\*\*\*\* were recorded for the solution which

TABLE I  
EFFICIENCY OF ELECTROLYTES IN FLOCCULATING SOIL COLLOIDAL SOLUTIONS

5 c.c. of Electrolyte N/5	1 Clay (sub- soil)	2 Miami Silt Loam	3 Clyde Silt Loam	4 Muck	5 Clay (soil)	6 Peaty Muck	7 Kaolin
1. HCl .....	*****	*****	*****	*****	*****	*****	*****
2. NaCl .....	*****	—	—	—	*****	—	*****
3. KCl .....	*****	***	***	—	*****	—	***
4. NH <sub>4</sub> Cl .....	*****	***	***	—	*****	—	*****
5. BaCl <sub>2</sub> .....	*****	*****	*****	*****	*****	*****	*****
6. CaCl <sub>2</sub> .....	*****	*****	*****	***	*****	***	*****
7. HgCl <sub>2</sub> .....	*****	—	—	—	***	—	—
8. MgCl <sub>2</sub> .....	*****	*****	*****	—	*****	*	*****
9. SnCl <sub>4</sub> .....	*****	***	***	***	*****	***	*****
10. HNO <sub>3</sub> .....	*****	*****	*****	*****	*****	*****	*****
11. NaNO <sub>3</sub> .....	*****	—	—	—	*****	—	*****
12. KNO <sub>3</sub> .....	*****	***	***	—	*****	—	*****
13. NH <sub>4</sub> NO <sub>3</sub> .....	*****	***	***	—	*****	—	*****

TABLE I—(Continued)

## EFFICIENCY OF ELECTROLYTES IN FLOCCULATING SOIL COLLOIDAL SOLUTIONS

5 c.c. of Electrolyte N/5	1 Clay (sub- soil)	2 Miami Silt Loam	3 Clyde Silt Loam	4 Muck	5 Clay (soil)	6 Peaty Muck	7 Kaolin
14. $\text{Ca}(\text{NO}_3)_2$	****	****	****	****	****	****	****
15. $\text{Hg}_2(\text{NO}_3)_2$	****	****	****	****	****	****	****
16. $\text{AgNO}_3$	****	****	****	****	****	****	****
17. $\text{Pb}(\text{NO}_3)_2$	****	****	****	****	****	****	****
18. $\text{H}_2\text{SO}_4$	****	****	****	****	****	****	****
19. $\text{KHSO}_4$	****	****	****	***	****	***	****
20. $(\text{NH}_4)_2\text{SO}_4$	****	—	—	—	****	—	****
21. $\text{K}_2\text{SO}_4$	****	****	***	—	****	—	****
22. $\text{K}_2\text{S}_2\text{O}_7$	****	****	****	***	****	***	****
23. $\text{MnSO}_4$	****	****	****	**	****	**	****
24. $\text{CuSO}_4$	****	****	****	***	****	***	****
25. $\text{FeSO}_4$	****	****	****	****	****	****	****
26. $\text{Fe}_2(\text{SO}_4)_3$	****	****	****	****	****	****	****
27. $\text{K}_2\text{S}$	****	***	***	—	****	—	—
28. $\text{NaSO}_3$	****	—	—	—	****	—	*
29. $\text{NaHSO}_3$	****	—	—	—	****	—	—
30. $\text{Na}_2\text{S}_2\text{O}_3$	****	—	—	—	****	—	—
31. $\text{AlK}(\text{SO}_4)_2$	****	****	****	****	****	****	****
32. $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2$	****	****	****	****	****	****	****
33. $\text{FeS}$	—	—	—	—	—	—	****
34. $\text{ZnS}$	—	—	—	—	—	—	—
35. $\text{NaOH}$	****	****	**	*	****	—	****
36. $\text{KOH}$	****	****	****	—	****	—	****
37. $\text{Ba}(\text{OH})_2$	****	****	****	****	****	****	****
38. $\text{MgO}$	****	—	—	—	****	—	****
39. $\text{CaO}$	****	****	****	****	****	****	****
40. $\text{H}_3\text{PO}_4$	****	****	****	****	****	****	****
41. $\text{NaH}_2\text{PO}_4$	****	—	—	—	****	—	***
42. $\text{CaH}_4(\text{PO}_4)_2$	***	—	—	—	*	—	—
43. $\text{Ca}_3(\text{PO}_4)_2$	—	—	—	—	—	—	—
44. $\text{KH}_2\text{PO}_4$	****	*	*	—	****	—	—
45. $\text{K}_2\text{HPO}_4$	****	*	**	—	****	—	—
46. $\text{K}_2\text{CO}_3$	****	***	***	—	****	—	—
47. $\text{Na}_2\text{CO}_3$	****	—	—	—	****	—	—
48. $\text{NaHCO}_3$	****	—	—	—	****	—	—
49. $\text{CaCO}_3$	—	—	—	—	—	—	—
50. $3\text{MgCO}_3\cdot\text{Mg}(\text{OH})_2$	****	—	—	—	****	—	***
51. $\text{FeCO}_3$	—	—	—	—	—	—	—
52. $(\text{NH}_4)_2\text{CO}_3$	****	—	—	—	****	—	—
53. $\text{CH}_3\text{COOH}$	****	****	***	—	****	—	—
54. $\text{NaC}_2\text{H}_3\text{O}_2$	****	—	—	—	****	—	****
55. $\text{Ca}(\text{C}_2\text{H}_3\text{O}_2)_2$	****	****	****	****	****	****	****
56. $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2$	****	****	****	****	****	****	****
57. $\text{C}_2\text{H}_3\text{O}_4$	****	***	***	***	****	***	****
58. $\text{C}_2\text{H}_3\text{O}_7$	****	****	****	****	****	****	****
59. $\text{C}_{17}\text{H}_{35}\text{COOH}$	—	—	—	—	—	—	—
60. $\text{As}_2\text{O}_3$	—	—	—	—	—	—	—
61. $(\text{NH}_4)_2\text{C}_2\text{O}_4$	****	****	****	—	****	—	*
62. $\text{NaKC}_4\text{H}_4\text{O}_6$	****	—	***	—	****	—	****
63. $\text{KBr}$	****	****	****	—	****	—	****
64. $\text{KI}$	****	****	****	—	****	—	****
65. $\text{KSCN}$	****	****	****	—	****	—	****
66. $\text{PbO}_2$	—	—	—	—	—	—	—
67. $\text{K}_2\text{Cr}_2\text{O}_7$	****	***	***	*	****	—	****



was flocculated the most. The one next in apparent efficiency was marked \*\*\*\*, and so on until a negative sign was used if no precipitate appears at the bottom of the test tube in 24 hours. Duplicate determinations were made in all of the experiments.

The results presented in Table I show that besides the familiar difference in efficiencies of different electrolytes with the same colloidal solution, the same electrolyte does not act alike with different suspensions, the easiest solutions to flocculate being that of clay and kaolin, followed by loams and, finally, mucks. This question is almost entirely overlooked by many soil investigators. As it was pointed out in the introductory remarks of this article, no studies have been recorded in soil literature, so far as the writer has been able to determine, on the flocculation of suspensions other than those of clay and kaolin. As a result, the conclusions regarding this process (perhaps as well as others) in soils have been based upon the results obtained from studies with a limited number of soils. But such conclusions, judging from the results presented in Table I may be erroneous, due undoubtedly to the fact that soils differ one from another in many respects, namely: chemically, physically and biologically. They may have different origin and different history with respect to their management. When taken from the same locality, as they were in this case, they may have only one factor in common, namely—climate. Very probably, a given type of soil, if exposed to different climatic conditions for a sufficient length of time, would behave differently with the same electrolyte. For instance, Lipman and Waynick (26) in a recently published article showed that the colloidal content of a Kansas soil, as judged from the suspended material after standing for 24 hours, was considerably modified by placing it in the climate either of California or of Maryland.

Strong acids are very good coagulants but they are not always better than some of their salts. This point is especially well brought forth by the next experiment. The salts of the heavy metals used have a much stronger flocculating power than those of lighter ones with respect to these soils. The trivalent cation is more efficient than a divalent one and this latter is better than a monovalent cation. Yet the tetravalent stannic chloride does not seem to do as efficient work as the divalents, barium chloride or calcium chloride. Contrary to the prevalent opinion, bases flocculate when used in this concentration. Only muck resists monovalent bases and yields fairly easily to divalents, both barium hydroxide and calcium hydroxide.

*Experiment II.* The minimum amount of electrolyte in solution required for the flocculation of a given amount of soil colloidal solution.

For this experiment all colloidal solutions were brought to as nearly the same concentration, as was possible. All stock colloidal solutions were so diluted that they contained .02735 gm. of dry material when 100

c.c. of solution was evaporated. To determine the minimum electrolyte requirement the following procedure was adopted.

Ten c.c. of colloidal solution was placed in each of a series of from 8 to 16 tubes, 25 c.c. graduated tubes being used as containers. Then, to the tube No. 1 was added 0.1 c.c. of N/5 salt solution; to No. 2, 0.2 c.c.; to No. 3, 0.3 c.c., etc. increasing gradually the amount of salt added. The solutions were vigorously shaken and allowed to stand over night. Now, if solutions in tubes Nos. 1, 2, and 3 have not settled while the rest of the solutions clarified, then .4 c.c. of that salt or acid was the requirement recorded. Often all solutions in a series was prepared with 15, 20, 25 or even 50 c.c. to which the small quantities of a flocculant were added. The recorded results, however, for convenience were all calculated to indicate the requirement per 10 c.c. of colloidal solution.

TABLE II  
MINIMUM ELECTROLYTE REQUIREMENT FOR COAGULATION OF 10 C.C. OF SOIL COLLOIDAL SOLUTIONS OF EQUAL CONCENTRATIONS

Electrolyte N/5	1 Clay (subsoil) c.c.	2 Miami Silt Loam c.c.	3 Clyde Silt Loam c.c.	4 Muck c.c.	5 Clay (soil) c.c.	6 Peaty Muck c.c.
HCl .....	.033	.100	.10	.200	.033	.20
BaCl <sub>2</sub> ..	.050	.100	.15	.300	.050	.40
CaCl <sub>2</sub> .....	.100	.200	.20	1.000	.100	1.50
MgCl <sub>2</sub> .....	.100	.500	.50	Negative with 10 c.c.	.100	Negative with 10 c.c.
SnCl <sub>4</sub> .....	.050	.100	.10	.200	.050	.15
HNO <sub>3</sub> .....	.050	.100	.10	.200	.050	2 0-3.0
Ca(NO <sub>3</sub> ) <sub>2</sub> .....	.100	.200	.20	2.000	.05- 1	2.0-3.0
Hg <sub>2</sub> (NO <sub>3</sub> ) <sub>2</sub> .....	.033	.033	.05	.050	.020	.10
AgNO <sub>3</sub> .....	.500	1.000	1.00	1.000	.300	...
Pb(NO <sub>3</sub> ) <sub>2</sub> .....	.020	.033	.05	.033	.020	.10
H <sub>2</sub> SO <sub>4</sub> .....	.050	.100	.15	.200	.050	.25
KHSO <sub>4</sub> .....	.100	.150	.20	.300	.100	.40
K <sub>2</sub> S <sub>2</sub> O <sub>7</sub> .....	.100	.150	.30	.500	...	.60
MnSO <sub>4</sub> .....	.033	.150	.15	1.500	...	...
CuSO <sub>4</sub> .....	.025	.050	.10	.100	...	...
FeSO <sub>4</sub> .....	.025	.150	.10	.300	...	...
Fe <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> .....	.025	.025	.05	.100	...	...
AlK(SO <sub>4</sub> ) <sub>2</sub> .....	.020	.020	.05	.100	...	...
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>6</sub> .....	0.25	.200	.15	1.500	...	...
NaOH .....	1.500	5.000	Negative with 10 c.c.	Negative with 12 c.c.	...	...
Ba(OH) <sub>2</sub> .....	.100	.150	.20	2.000	...	...
Ca(OH) <sub>2</sub> .....	.200	.300	1.00	2.000	...	...
H <sub>3</sub> PO <sub>4</sub> .....	.100	.200	.30	1.000	...	...
Ca(C <sub>2</sub> H <sub>3</sub> O <sub>2</sub> ) <sub>2</sub> .....	.050	.150	.15	2.000	...	...
Pb(C <sub>2</sub> H <sub>3</sub> O <sub>2</sub> ) <sub>2</sub> .....	.020	.025	.05	.035	...	...
C <sub>2</sub> H <sub>3</sub> O <sub>4</sub> .....	2.000	...	...	.500	...	...
C <sub>6</sub> H <sub>5</sub> O <sub>7</sub> .....	.150	1.500	2.00	1.000	...	...
(NH <sub>4</sub> ) <sub>2</sub> CO <sub>3</sub> .....	3.000	...	Negative	with 10 c.c.	...	...

The results presented in Table II and figure 1 show the difference in the efficiency of different electrolytes with different soils much better than the qualitative results in the Table I. The trivalent ferric sulphate and

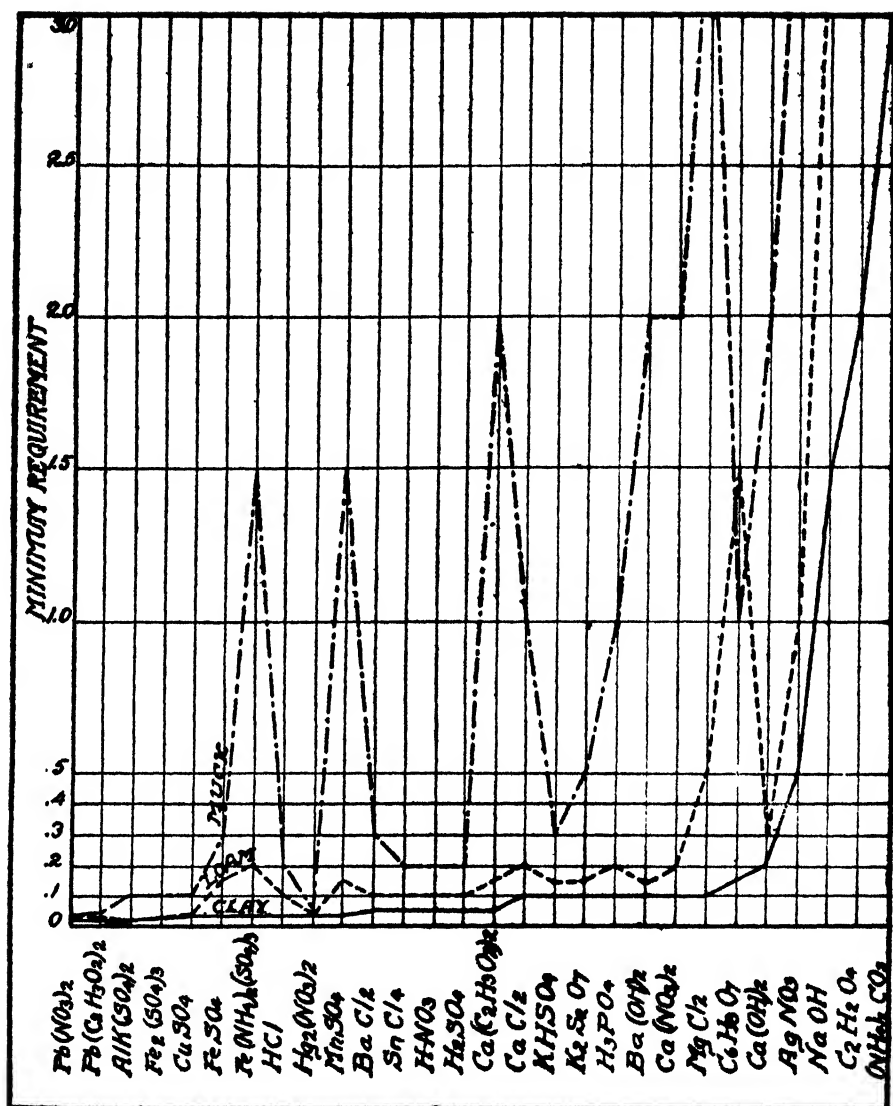


Fig. 1.—The minimum electrolyte requirement for coagulation of soil colloidal solutions.

aluminum potassium sulphate are not the leading ones; the salts of lead, being only divalent, both nitrate and acetate act better, especially in the case with muck solutions. There is not the slightest indication of following the formula of Schulze (37). As the chart shows, the silt is more resistant to the action of electrolytes than clay, and muck is the most resistant of the three selected classes. There is one striking fact brought out by this chart. With the best coagulants the minimum electrolyte re-

quirement of all solutions is nearly the same, as one notices in the cases of  $\text{Pb}(\text{NO}_3)_2$ ,  $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2$ ,  $\text{Hg}_2(\text{NO}_3)_2$  and to some extent  $\text{Fe}_2(\text{SO}_4)_3$  and  $\text{AlK}(\text{SO}_4)_2$ , but with others the variations are great and often very irregular, evidently being dependent not only upon the cation but also upon the anion, the chemical composition of the colloidal particles and the salts present in the original solutions. Undoubtedly, an important rôle is played by the so-called humic substances of the soil whose protective action was suggested by Fickendey (15) and later by Keppeler and Spangenberg (24); and perhaps the similar observations mislead Lyon, Fippin and Buckman (27) to make the statement that "the gelatinous colloids of the soil, such as some of the humic materials, are not agglutinated by the addition of electrolytes."

In order to ascertain to what extent this difference in resistance of colloidal solution to the flocculating action of electrolyte could be ascribed to the protective influence of humic material, an experiment was undertaken and the following obtained results may typify the case.

*Experiment III.* Effect of muck colloidal solution on the stability of clay colloidal solution.

The clay colloidal solution was mixed with muck colloidal solution in proportions from 100 per cent to 0 per cent of clay. The minimum electrolyte requirement of these resultant solutions was determined in the usual way. Both clay and muck suspensions were freshly prepared and the dry matter in both of them, as well as in the mixtures, was determined.

TABLE III  
EFFECT OF ORGANIC MATTER ON THE MINIMUM ELECTROLYTE REQUIREMENTS FOR COAGULATION OF CLAY COLLOIDAL SOLUTION

Clay Solution	Muck Solution	Dry weight per 100 c.c. of sol.	Electrolyte Requirement	
			$\text{Ca}(\text{OH})_2$ satur. at 20° C. per 10 c.c. of sol.	$\text{HNO}_3$ n/5 per 10 c.c. of sol.
100	0	.0730	.3 c.c.	.05 c.c.
75	25	.0578	.4 c.c.	.10 c.c.
50	50	.0411	.6 c.c.	.15 c.c.
25	75	.0261	1.4 c.c.	.20 c.c.
0	100	.0105	2.8 c.c.	.25 c.c.

The figures in Table III leave no doubt regarding the influence of organic material upon the stability of the solution. That the difference in stability of the colloidal solutions in the foregoing experiment was not due to the difference in their solid material content, but rather regardless of it, is absolutely proved by the next experiment.

*Experiment IV.* Effect of solid material present on the stability of soil colloidal solution.

The original stock solution of clay from Experiment I, was diluted 2, 8, and 32 times and the minimum coagulant requirement of each solution was determined.

TABLE IV  
EFFECT OF THE CONCENTRATION OF CLAY COLLOIDAL SOLUTION ON THE  
MINIMUM ELECTROLYTE REQUIREMENT

Concentration per 100 c.c. of solution. Relation	Gm.	CaCl <sub>2</sub> n/25 c.c.	Ca(NO <sub>3</sub> ) <sub>2</sub> n/25	Ca(OH) <sub>2</sub> saturated	CaSO <sub>4</sub> saturated	H <sub>2</sub> SO <sub>4</sub> n/25	AlK(SO <sub>4</sub> ) <sub>2</sub> n/25	KHSO <sub>4</sub> n/5	K <sub>2</sub> SO <sub>4</sub> n/5	FeSO <sub>4</sub> n/25	HNO <sub>3</sub> n/25
1	.18165	.3-.4	.3-.4	.3-.4	.3-.4	.5	.4	.2	.9	.4	.4
1/3	.04541	.3	.3	.2	.3	.3	.2	.1	.6	.15	.2-.3
1/16	.01135	.2	.2	.2	.2	.3	.1	.1	.4	.1	.2

Muck colloidal solution was freshly prepared, a portion of which was diluted to 1/3 of its original concentration, and the electrolyte requirement per 10 c.c of each solution follows:

TABLE IV—A  
EFFECT OF THE CONCENTRATION OF MUCK COLLOIDAL SOLUTION ON THE  
MINIMUM ELECTROLYTE REQUIREMENT

Electrolyte n/5	Original	1/3 of Original
AlK(SO <sub>4</sub> ) <sub>2</sub> .....	0 10 c.c.	0.050 c.c.
Fe <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> .....	0.10 c.c.	0.050 c.c.
Ph(NO <sub>3</sub> ) <sub>2</sub> .....	0 05 c.c.	0.038 c.c.

The results indicate very plainly, first, that with the decrease of concentration of colloidal solution the minimum electrolyte requirement for flocculation of that solution decreases also. For the solutions used this is true without exception. Second, the decrease in the minimum coagulant requirement is not proportional to the decrease in concentration of colloidal solution. This lack of proportionality is probably due to the mechanical difficulties of bringing the particles together to form an aggregate large enough for stopping the Brownian movement, since there are less chances for particles to strike a certain number of particles in a dilute solution than in a concentrated one.

There is a great deal of speculation regarding the nature of coagulation. Some authors describe it as a purely physical phenomenon, while others seem to favor the application of chemical laws to the same effect observed. A few examples will illustrate the point.

Whitney (43) explains the flocculation by means of surface tension. Using his own words: "If the potential of the surface particle of water is less than of a particle in the interior of the mass of liquid there will be surface tension and the two grains will not come together, because they would enlarge the surface area and increase the number of surface particles of the liquid. If, on the other hand, the potential of the particle on the surface of the liquid is greater than the potential of a particle in the interior of the liquid mass, the surface will tend to enlarge and the grains

of clay may come close together and be held there with some force, as their close contact increases the number of surface particles in the liquid around them. This probably explains the phenomenon of flocculation."

Quinke (34) later proposed a similar theory employing the change in surface tension between liquid and the oily substances, around the solid particles. Bary (7) thought that liquid penetrates the solid particles and the attraction between the two balances itself against the elasticity of the solid and the surface tension. Upon the addition of an electrolyte the osmotic pressure is changed, causing the withdrawal of water from the colloidal particles and coagulation results. Bancroft (3,4) in his recently published articles, summarizing the most important investigations on the subject, comes to the conclusion that in coagulation the adsorption is taking place only at the surfaces of the solid particles.

Duclaux (11), on the other hand, considers the colloids as electrolytes with the power of ionization and, although, the stability of colloidal solution is based on the equilibrium between the intermicellar liquid and the colloidal particles proper, yet the disturbance of this equilibrium implies the chemical change. Jordis (22) also attributes the coagulation to the chemical action. The similar view is held by Ashley (2). Arrhenius (1) noticed a close analogy between agglutination and the precipitation and concluded their nature to be the same, i. e. the chemical. The recent work of Beam and Eastlack (8) on the electrical synthesis of colloids shows that in the preparation of the hydrosols there is a very close association between the colloidal particles and the ions of some electrolyte, which give the stability to that hydrosol. In order to destroy the stability, or to bring about a coagulation, there is necessary more than a mere physical change.

The following experiment, which suggested itself by an accident, seems to throw some light upon the phenomenon of flocculation.

*Experiment V.* Effect of concentration of colloidal solution on the time required for coagulation, the amount of electrolyte added remaining the same.

In this experiment the clay and muck colloidal solutions were diluted to  $\frac{1}{2}$ ,  $\frac{1}{4}$ ,  $\frac{1}{8}$ , etc., of their original concentrations. To 5 c.c. of each of the resultant solutions was added 5 c.c. of electrolyte N/5, vigorously shaken for a few seconds and set aside. The time in minutes when the first floccules could be observed was recorded in Table V.

The results reveal a striking regularity of time requirement by differently diluted colloidal solutions. With the exceptions of the most concentrated solutions and the minor discrepancies in a few cases, the time necessary for flocculation is nearly inversely proportional to the concentration of that colloidal solution, or it is a splendid demonstration of the mass action law stating that "the velocity of a chemical reaction is pro-

portional to the quantities present in condition to react." In our case the amount of electrolyte added was the same in all cases and always present in abundance, while another component, the colloid solution, varied, and, being a limiting factor, altered the velocity of reaction.

TABLE V  
EFFECT OF THE CONCENTRATION OF COLLOIDAL SOLUTION ON THE TIME  
REQUIRED FOR COAGULATION  
CLAY COLLOIDAL SOLUTION

Concentration of colloid solution per 100 c.c.		.3633		.18165		.090825		.0454		.0227		.01135	
Electrolyte used  N/5	Temp. Degrees C.	Observed min.	Calculated	Observed min.	Calculated	Observed min.	Calculated	Observed min.	Calculated	Observed min.	Calculated	Observed min.	Calculated
HCl .....	21.9	0.5	3.50	4.5	.70	14.0	14.0	31.0	27.5	66 0	55.0	109	109
H <sub>2</sub> SO <sub>4</sub> .....	25.0	0.5	1.90	3.5	3.75	9 0	7.5	17.0	15.0	32.0	30.0	60	60
HNO <sub>3</sub> .....	21.0	0.5	5.60	4.0	11.26	11.0	12.5	28.0	25.0	55.0	50.0	100	100
H <sub>3</sub> PO <sub>4</sub> .....	21.6	1.0	3.75	6.0	7.50	13.0	15.0	30.0	30 0	64.0	60.0	120	120
CH <sub>3</sub> COOH .....	22.0	1.0	3.50	7.0	7.00	17.0	14.0	30.0	27.5	60.0	55.0	110	110
Cr <sub>2</sub> H <sub>2</sub> O <sub>4</sub> .....	21.0	2.5	8.80	7.0	17.50	26.0	35.0	52.0	70.0	140.0	140 0	...	...
C <sub>6</sub> H <sub>8</sub> O <sub>7</sub> .....	21.0	0.5	3.10	1.5	6.30	11.0	12.5	21.0	25.0	50.0	50.0	100	100
CaCl <sub>2</sub> .....	23.7	0.5	2.00	4.0	4.00	11.0	8.0	26 0	16 0	38.0	32.0	64	64
Ca(NO <sub>3</sub> ) <sub>2</sub> .....	19.2	1.0	2.80	5.0	5.60	13.0	11.1	29.0	22.2	45.0	44.5	89	89
FeSO <sub>4</sub> .....	20.8	3.0	3.12	6.5	6.25	13.0	12.5	24.0	25 0	54.0	50 0	100	100
KOH .....	20.8	3.0	4.00	9.0	8.00	21.0	16.1	39.0	32.2	57.0	64.5	129	129

TABLE V—A  
MUCK COLLOIDAL SOLUTION

		Original		½ Original		¼ Original		¼ Original	
AlK(SO <sub>4</sub> ) <sub>2</sub>	20.2	8	13	24	26	53	51.5	103	103
Fe <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub>	21.0	3	11.	11	21.5	23	43	86	86
Pb(NO <sub>3</sub> ) <sub>2</sub>	21.4	1½	2½	4	5	10	10.5	21	21

The figures on the right side of the column are calculated, taking the result of the most dilute solution for a basis. The close agreement between the results observed and the theoretical values is still better demonstrated by figures 2 and 3.

Taking into consideration the fact that the reactions were allowed to take place at room temperature, which necessarily fluctuated in the course of time needed for the completeness of experiment with each electrolyte studied, one notices the close coincidence of the two lines, which seem to indicate that there is a close relation between the chemical reactions and the reaction between the electrolyte and the colloidal particles or, rather, the ions associated with those particles. However, whether a flocculation is a chemical reaction, or a reaction that only obeys the chemical law is more than we can say from the results thus far at our disposal.

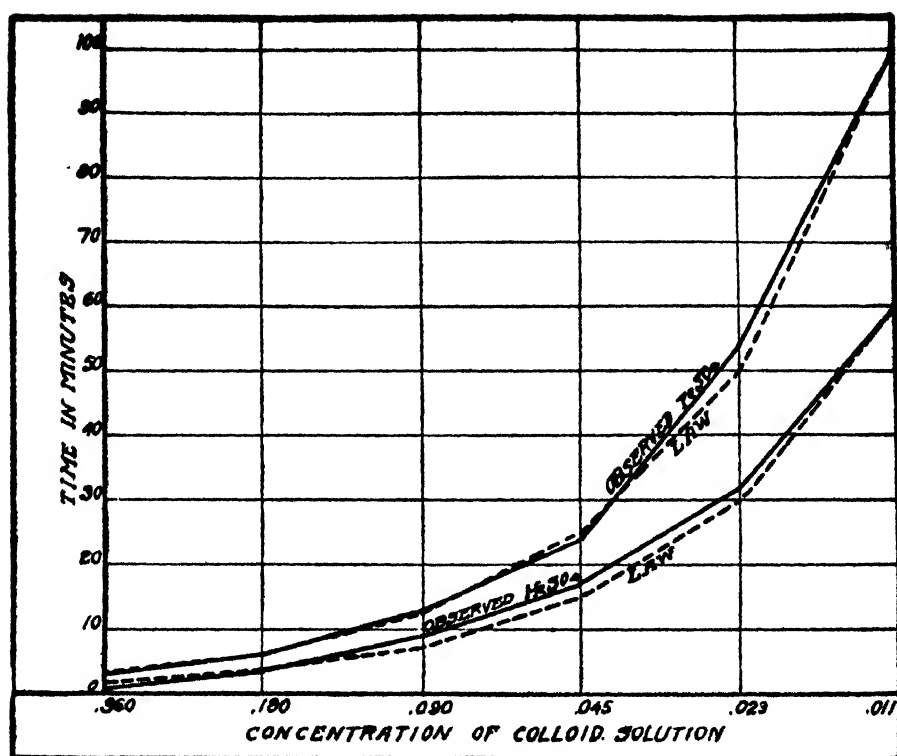


Fig. 2.—The relation between the Mass Action Law and the flocculation of clay colloidal solution.

#### SUMMARY

1. Besides the fact that the flocculating efficiency of different electrolytes with the same colloidal solution is different, the results show that the efficiency of the same electrolyte with the solutions from different soils varies considerably, depending largely upon the chemical composition of the soils.

2. Schulze's valency law does not hold true with the soil colloidal solutions studied.

3. Humic materials hinder the coagulating power of the electrolytes.

4. It takes a greater amount of electrolyte for flocculation of a more concentrated soil colloidal solution than that for a less concentrated one.

5. In the flocculation of the soil colloidal solutions by the electrolyte, the reaction obeys, within the experimental error, the law of mass action.

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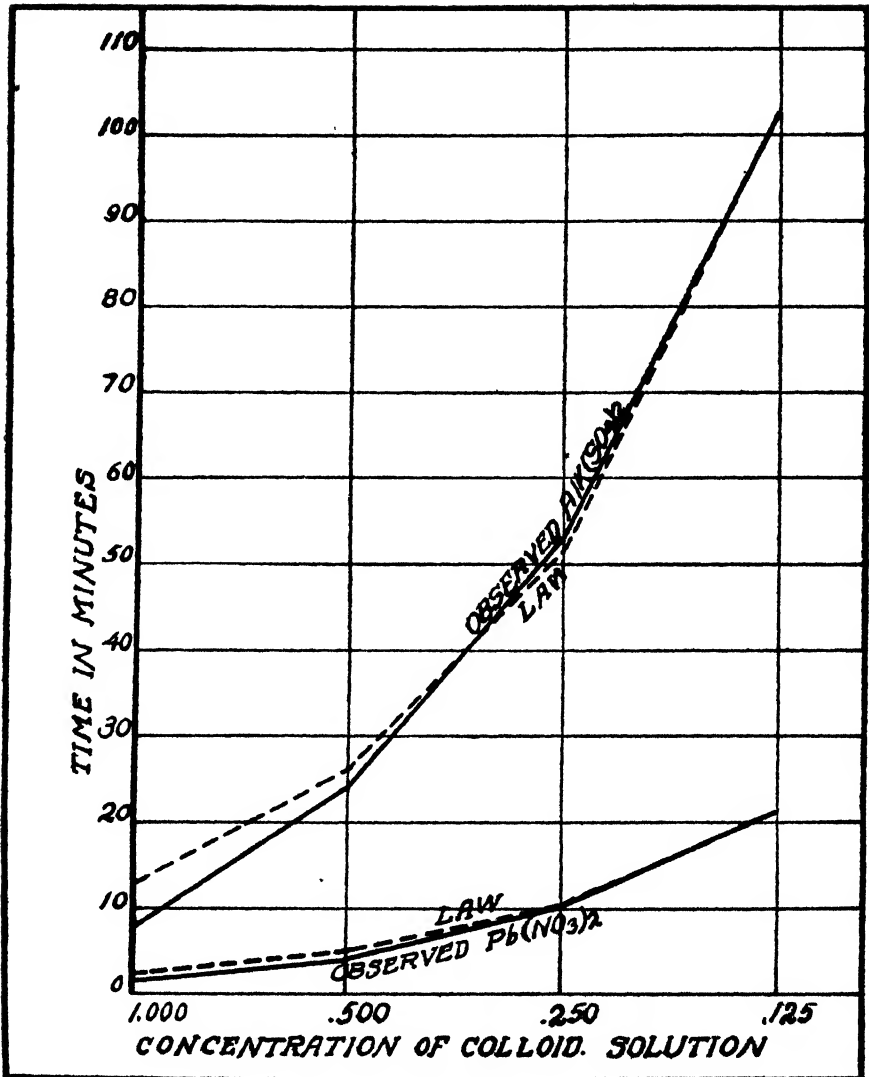


Fig. 3.—The relation between the Mass Action Law and flocculation of muck colloidal solution.

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